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(54) Title: DNA ENCODING 5-HT$_4$ SEROTONIN RECEPTORS AND USES THEREOF

(57) Abstract

This invention provides an isolated nucleic acid molecule encoding a mammalian 5-HT$_4$ receptor and an isolated nucleic acid molecule encoding a human 5-HT$_4$ receptor, an isolated protein which is a mammalian 5-HT$_4$ receptor, an isolated protein which is a human 5-HT$_4$ receptor, vectors comprising an isolated nucleic acid molecule encoding a mammalian 5-HT$_4$ receptor, vectors comprising an isolated nucleic acid molecule encoding a human 5-HT$_4$ receptor, mammalian cells comprising such vectors, antibodies directed to the 5-HT$_4$ receptor, nucleic acid probes useful for detecting nucleic acid encoding a mammalian or human 5-HT$_4$ receptor, antisense oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a mammalian or human 5-HT$_4$ receptor, pharmaceutical compounds related to the human 5-HT$_4$ receptor, and nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian or human 5-HT$_4$ receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatments for alleviating abnormalities associated with a human 5-HT$_4$ receptor.

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DNA ENCODING 5-HT, SEROTONIN RECEPTORS AND USES THEREOF

Background of the Invention

Throughout this application various publications are referred to by partial citations within parenthesis. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications, in their entireties, are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Molecular cloning efforts have provided primary amino acid sequence and signal transduction data for a large collection of serotonin receptor subtypes. These include five cloned 5-HT_1-like receptors, three cloned 5-HT_2 receptors, and one 5-HT_3 receptor. The 5-HT_1 subfamily includes: 5-HT_{1A} (Fargin, 1988; Kobilka, 1989), 5-HT_{1B}/5-$HT_{1D\beta}$ (Weinshank et al., 1991; Demchyshyn et al., 1992; Jin et al., 1992; Adham et al., 1992; Maroteaux et al., 1992; Voight et al., 1991), 5-$HT_{1D\alpha}$ (Branchek et al. 1991; Hamblin and Metcalf, 1991; Weinshank et al., 1992), 5-HT_{1E} (Levy et al., 1992; McAllister et al., 1992; Zgombick et al., 1992) and 5-HT_{1F} (Adham et al., 1993). All five have been shown to couple to the inhibition of adenylate cyclase activity. The 5-HT_2 family includes the 5-HT_2 receptor (Pritchett et al., 1988), 5-HT_{1C} (Julius et al., 1989) and 5-HT_{2F} (Rat Stomach Fundus; Foquet et al., 1992; Kursar et al., 1992). These receptors all couple to phosphoinositide hydrolysis. The 5-HT_3 receptor is a ligand-gated ion channel (Maricq et al., 1991).

Alth ugh this work represents enormous success, the absence of molecular biological information on the 5-

HT_4 receptors, which have been shown in native tissues to couple to the activation of adenylate cyclase as a primary mode of signal transduction (Dumius et al., 1988; Bockaert et al., 1990), is apparent. In a previous copending application (U.S. Serial No., 971,690, filed November 3, 1992), we reported the cloning of the first mammalian 5-HT receptor that couples to the stimulation of adenylate cyclase activity which we named 5-HT_{4B}. The 5-HT_{4B} receptor was subsequently renamed to the "5-HT_7 receptor" by the "Serotonin Receptor Nomenclature Committee" of the IUPHAR. The pharmacological properties of this receptor indicated that it was similar to a series of functionally defined 5-HT receptors described in the porcine vena cava (Trevethick et al., 1984), cat saphenous vein, coronary arteries (Cushing and Cohen, 1992), and several vascular dilatory effects (Mylecharane and Phillips, 1989). However, the classically defined 5-HT_4 receptor remained to be cloned. We now report the cloning of the pharmacologically-defined 5-HT_4 receptor which we have previously called 5-HT_{4A} and now designate as the 5-HT_4 receptor. This receptor also stimulates adenylate cyclase activity but unlike 5-HT_{4B}, is sensitive to a series of benzamide derivatives which act as agonists or partial agonists at this subtype. The presence of this subtype in the brain, particularly in areas such as the hippocampus, indicates a potential role in cognitive enhancement. In addition, the 5-HT_4 receptor has been described functionally in the heart, adrenal, bladder, and alimentary canal indicating potential roles in achalasia, hiatal hernia, esophageal spasm, irritable bowel disease, postoperative ileus, diabetic gastroparesis, emesis and other diseases of the gastrointestinal tract, as well as in cardiac, urinary, and endocrine function.

Summary of the Invention

This invention provides an isolated nucleic acid molecule encoding a mammalian 5-HT_4 receptor. In a preferred embodiment of this invention, the isolated nucleic acid encodes a human 5-HT_4 receptor. In another embodiment of this invention, the nucleic acid molecule encoding a human 5-HT_4 receptor comprises a plasmid designated pBluescript-hS10 (ATCC Accession No. 75392). In another embodiment of this invention a nucleic acid molecule encoding a mammalian 5-HT_4 receptor comprises a plasmid designated pcEXV-S10-87 (ATCC Accession No. 75390). In another embodiment of this invention a nucleic acid molecule encoding a mammalian 5-HT_4 receptor comprises a plasmid designated pcEXV-S10-95 (ATCC Accession No. 75391).

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian 5-HT_4 receptor. This invention also provides a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT_4 receptor.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a mammalian 5-HT_4 receptor so as to prevent translation of the mRNA molecule. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a human 5-HT_4 receptor so as to prevent translation of the mRNA molecule.

This invention provid s a monoclonal antibody directed to a mammalian 5-HT_4 recept r. This inventi n also provides a monoclonal antibody directed to a human 5-HT_4 receptor.

This invention provides a pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a mammalian 5-HT_4 receptor and a pharmaceutically acceptable carrier. This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of mammalian 5-HT_4 receptor and a pharmaceutically acceptable carrier.

This invention provides a pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human 5-HT_4 receptor and a pharmaceutically acceptable carrier. This invention also provides pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a human 5-HT_4 receptor and a pharmaceutically acceptable carrier.

This invention provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian 5-HT_4 receptor so positioned within such genome as to be transcribed into antisense mRNA complementary to mRNA encoding the mammalian 5-HT_4 receptor and when hybridized to mRNA encoding the mammalian 5-HT_4 receptor, the complementary mRNA reduces the translation of the mRNA encoding the mammalian 5-HT_4 receptor.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human 5-HT_4 so positioned within such genome as to be transcribed into antisense mRNA complementary to mRNA encoding the human 5-HT_4 and when hybridized to mRNA encoding the human 5-HT_4, the complementary mRNA reduces the translation of the mRNA encoding the human 5-HT_4.

This invention provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian 5-HT_4 receptor so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the mammalian 5-HT_4 receptor and when hybridized to mRNA encoding the 5-HT_4 receptor, the antisense mRNA thereby prevents the translation of mRNA encoding the 5-HT_4 receptor.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human 5-HT_4 receptor so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the human 5-HT_4 receptor and when hybridized to mRNA encoding the human 5-HT_4 receptor, the antisense mRNA thereby prevents the translation of mRNA encoding the human 5-HT_4 receptor.

This invention also provides a method of determining the physiological effects of expressing varying levels of a mammalian 5-HT_4 receptor which comprises producing a transgenic nonhuman animal whose levels of mammalian 5-HT_4 receptor expression are varied by use of an inducible promoter which regulates mammalian 5-HT_4 receptor expression.

This invention also provides a method of determining the physiological effects of expressing varying lev ls

of a human 5-HT_4 receptor which comprises producing a transgenic nonhuman animal whose levels of human 5-HT_4 receptor expression ar varied by use of an inducible promoter which regulates human 5-HT_4 receptor expression.

This invention further provides a method of determining the physiological effects of expressing varying levels of mammalian 5-HT_4 receptor which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of mammalian 5-HT_4 receptor.

This invention further provides a method of determining the physiological effects of expressing varying levels of human 5-HT_4 receptor which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human 5-HT_4 receptor.

This invention provides a method for determining whether a compound not known to be capable of specifically binding to a human 5-HT_4 receptor can specifically bind to the human 5-HT_4 receptor, which comprises contacting a mammalian cell comprising a plasmid adapted for expression in a mammalian cell which plasmid further comprises a DNA which expresses a human 5-HT_4 receptor on the cell's surface with the compound under conditions permitting binding of ligands known to bind to a human 5-HT_4 receptor, detecting the presence of any compound bound to the human 5-HT_4 receptor, the presence of bound compound indicating that the compound is capable of specifically binding to the human 5-HT_4 receptor.

This invention provides a method of screening drugs to identify drugs which interact with, and specifically bind to, a human 5-HT_4 receptor on the surface of a cell, which comprises contacting a mammalian cell which

comprises a plasmid adapted for expression in a mammalian cell which plasmid further comprises DNA which expresses a human 5-HT_4 receptor on the cell's surface with a plurality of drugs, determining those drugs which bind to the human 5-HT_4 receptor expressed on the cell surface of the mammalian cell, and thereby identifying drugs which interact with, and specifically bind to, the human 5-HT_4 receptor.

This invention provides a method for identifying a compound which specifically binds to and activates or blocks the activation of a human 5-HT_4 receptor on the surface of a mammalian cell, which comprises contacting the mammalian cell which comprises a plasmid adapted for expression in the mammalian cell such plasmid further comprising DNA which expresses the human 5-HT_4 receptor on the cell surface of the mammalian cell with the compound, determining whether the compound activates or blocks the activation of the human 5-HT_4 receptor and thereby identifying the compound as a compound which binds to, and activates or blocks the activation of the human 5-HT_4 receptor.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a human 5-HT_4 receptor allele which comprises: a.) obtaining DNA of subjects suffering from the disorder; b.) performing a restriction digest of the DNA with a panel of restriction enzymes; c.) electrophoretically separating the resulting DNA fragments on a sizing gel; d.) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a 5-HT_4 receptor and labelled with a detectable marker; e.) detecting labelled bands which have hybridized to the DNA enc ding a 5-HT_4 receptor labelled with a detectable marker to creat a unique band pattern specific to the

DNA of subjects suffering fr m the disorder; f.) preparing DNA obtained for diagnosis by st ps a-e; and g.) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

Brief Description f Figur s

Figure 1: Nucleotide and corresponding amin acid sequence of the S10-87 cDNA clone. Only partial 5′ and 3′ untranslated sequences are shown.

Figure 2: Nucleotide and corresponding amino acid sequence of the S10-95 cDNA clone. Only partial 5′ and 3′ untranslated sequences are shown.

Figure 3: Comparison of amino acid sequences between clones S10-87 (top row) and S10-95 (bottom row). The overall homology is 96.7%.

Figure 4: Comparison of the rat S10 receptor deduced amino acid sequences with those of other serotonin receptors and with the canine histamine H2 receptor. Solid bars, the seven putative membrane-spanning domains (TM I-VII). Shading, homologies between the S10 receptors and other receptors. Hp78, 5-HT4B or hp78a receptor (U.S. Serial No., 971,960, filed, November 3, 1992, copending).

Figure 5: Nucleotide and amino acid sequences of the human S10 PCR clone. The numbering is given according to the rat S10-95 clone.

Figure 6: Comparison of nucleotide sequences between the human PCR S10 clone and the rat S10 cDNA clone. Top row: human sequence, the numbering is given according to the rat S10 nucleotide sequence. The bottom row outlines differences in the rat sequence (overall homology: 90.7%).

Figure 7: Comparison of deduced amino acid sequences between the Human S10 PCR clone and the rat S10 cDNA clone. Top row: human S10 sequence, the numbering is

given according to the rat S10 amino acid sequence. Th bott m r w outlines differences in the rat sequence (overall homology: 92.3 %).

Figure 8: Comparison of binding affinities of key compounds at the S10 clone with adenylate cyclase functional responses obtained with mouse collicular neurons. A correlation plot was constructed between affinity constants of drugs for the S10 receptor with those obtained at a pharmacologically defined 5-HT_4 receptor. Binding values for the correlation were taken from table 1 and were expressed as the negative logarithm. Functional data were taken from Dumuis et al. (1988). The correlation coefficient calculated by linear regression was 0.96 indicating that the rank order of potency for the compounds was similar in both preparations.

Figure 9: Stimulation of cAMP production by 5-HT in transiently transfected Cos-7 cells expressing the cloned rat 5-HT_4 (CG-7) receptor and antagonism by ICS 205930. cAMP measurements on intact cells were as described under Methods and Materials. Each data point represents the mean of triplicates from a single experiment representative of at least 2 others. The vertical bars indicate S.E.M. Data are presented as percent maximum cAMP released by 5-HT (basal cAMP release: 0.020 ± 0.002 pmol/ml/10 min; maximum cAMP release: 0.42 ± 0.03 pmol/ml/10 min).

Figure 10: Stimulation of cAMP production by 5-HT in transiently transfected Cos-7 cells expressing the cloned rat 5-HT_4 (CG-8) receptor and antagonism by ICS 205930. cAMP measurements on intact cells were as described under Methods and Materials. Each data point represents the mean of triplicates from a single experiment representative of at least two others. The

vertical bars indicate S.E.M. Data are presented as percent maximum cAMP r leased by 5-HT (basal cAMP rel ase: 0.023 ± 0.004 pmol/ml/10 min; maximum cAMP release: 0.57 ± 0.04 pmol/ml/10 min).

Figure 11A: Nucleotide sequence of the partial human S10-87 clone. Only partial 3' untranslated sequences are shown (SEQ. ID NO. 14).

Figure 11B: Deduced amino acid sequence encoded by the nucleotide sequence of Figure 11A of the partial human S10-87 clone (SEQ. ID NO. 15).

Figure 12: Comparison of the nucleotide sequences between the human (top row) and the rat S10-87 (bottom row) cDNA clones. The overall identity is 90.8%.

Figure 13: Comparison of the deduced amino acid sequences between the human (top row) and the rat (bottom row) S10-87 receptors. The overall identity is 93.9%.

Figure 14A: Nucleotide sequence of the full length human S10-95 clone (SEQ. ID NO. 7).

Figure 14B: Deduced amino acid sequence encoded by the nucleotide sequence of Figure 14A (SEQ. ID NO. 8).

Figure 15: Comparison of the nucleotide sequences between the human (top row) and the rat (bottom row) S10-95 cDNA clones. The overall identity is 90.7%.

Figure 16: Comparison of the deduced amino acid sequences between the human (top row) and the rat (bottom row) S10-95 receptors. The overall identity is 93.8%.

Figure 17: Comparison of the nucleotide sequences corresponding to th available coding regions between the tw human isoforms (top row S10-95; bottom row S10-87) of the 5-HT_4 receptor. The overall identity is 92%.

Figure 18: Comparison of the deduced amino acid sequences between the two human isoforms (top row S10-95; bottom row S10-87) of the 5-HT_4 receptor. The overall identity is 90%.

Figure 19: Inhibition of [^{3}H]GR11380 binding on the cloned rat CG-8 receptor by 5-HT, in the absence and presence of Gpp(NH)p (100 μM). Membranes harvested from transient transfectants (COS-7 cells) were incubated with [^{3}H]IGR113808 (0.2-0.4 nM) for 30 min at 37°C. Nonspecific binding was defined by 50 μM unlabelled 5-HT. Data are from a single experiment. Data were analyzed by computer-assisted nonlinear regression analysis (Accufit; Lundon Software).

Detailed Description of the Invention

This invention provides an isolated nucleic acid molecule encoding a mammalian 5-HT_4 receptor. This invention further provides an isolated nucleic acid molecule encoding a human 5-HT_4 receptor. As used herein, the term "isolated nucleic acid molecule" means a non-naturally occurring nucleic acid molecule that is, a molecule in a form which does not occur in nature. Examples of such an isolated nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a mammalian 5-HT_4 receptor or a human 5-HT_4 receptor. As used herein, "5-HT_4 receptor" means a molecule which, under physiologic conditions, is substantially specific for the neurotransmitter serotonin, is saturable, of high affinity for serotonin and the activation of which is coupled to the activation of adenylate cyclase and the "5-HT_4 receptor" is also sensitive to benzamide derivatives which act as agonists and partial agonists at this receptor subtype. One embodiment of this invention is an isolated nucleic acid molecule encoding a mammalian 5-HT_4 receptor. Such a molecule may have coding sequences substantially the same as the coding sequences shown in Figures 1 and 2 and 5 (SEQ ID NOs. 1, 3 and 5). A preferred embodiment is an isolated nucleic acid molecule encoding a human 5-HT_4 receptor. Such a molecule may have a coding sequence substantially the same as the coding sequence shown in Figure 5 (SEQ ID NO. 5). The DNA molecules of Figures 1, 2 and 5 (Seq ID NOs. 1, 3 and 5) encode the sequence of mammalian 5-HT_4 receptors. The DNA molecule of Figure 5 (Seq ID No. 5) encodes a human 5-HT_4 receptor. This invention further provides isolated DNA molecules encoding mammalian 5-HT_4 receptors having the sequence H_2N-Y-X-COOH wherein Y is the amino acid sequence beginning at amino acid 1 and ending at amin

acid 359 of Figure 1 (SEQ ID NOs. 1 and 2) and wherein X is an amino acid sequenc encoding the carb xy terminal r gi n of the receptor. The nucleic acid molecules of Figures 1 and 2 (SEQ ID NOs 1-4) encode 5-HT_4 receptors having an identical sequence Y and differing only in their carboxy terminal region X beginning at amino acid 360. One means of isolating a nucleic acid molecule encoding a mammalian 5-HT_4 receptor is to probe a mammalian genomic library with a natural or artificially designed DNA probe, using methods well known in the art. In the preferred embodiment of this invention, the mammalian 5-HT_4 receptor is a human protein and the nucleic acid molecule encoding the human 5-HT_4 receptor is isolated from human cDNA. Degenerate oligonucleotide primers derived from transmembrane (TM) domains of 5-HT_{1A}, 5-HT_{1C}, 5-HT_2 and 5-$HT_{10\alpha/\beta}$ receptors are useful for identifying cDNA containing a nucleic acid molecule encoding a 5-HT_4 receptor, obtaining a probe specific to a mammalian 5-HT_4 receptor and for isolating a nucleic acid molecule encoding a mammalian 5-HT_4 receptor.

DNA and cDNA molecules which encode a mammalian 5-HT_4 receptor are used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5′ untranslated region of the isolated clone, and other stability, processing, transcription, translation, and tissue specificity determining regions from the 3′ and 5′ untranslated regions of the isolated gene are thereby obtained.

This invention provides an isolated nucleic acid

molecule which has a nucleic acid sequence which differs from the sequence of a nucleic acid molecule encoding a 5-HT_4 receptor at one or more nucleotides and which does not encode a protein having 5-HT_4 receptor activity. As used herein, "5-HT_4 receptor activity" means the capability of receptor to specifically bind the neurotransmitter, serotonin under physiological conditions and the capability of the receptor to activate adenylate cyclase when the receptor is coupled to adenylate cyclase. An example of a isolated nucleic acid molecule provided by this invention is a nucleic acid molecule which has an in-frame stop codon inserted into the coding sequence such that the transcribed RNA is not translated into protein.

This invention further provides a cDNA molecule encoding a mammalian 5-HT_4 receptor, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figures 1, 2 and 5 (Seq ID NOs. 1, 3 and 5). This invention provides a cDNA molecule encoding a human 5-HT_4 receptor, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figure 5 (SEQ ID NO. 5). These molecules and their equivalents were obtained by the means described above.

This invention also provides an isolated protein which is a mammalian 5-HT_4 receptor. In a preferred embodiment of this invention, the protein is a human 5-HT_4 receptor protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figures 1, 2 and 5 (SEQ ID Nos. 1-6). In another embodiment of this invention, the protein is a murine 5-HT_4 receptor protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figures 1, 2 and 5 (SEQ ID NOs. 1-6). As used

herein, the term "isolated protein" is intended to encompass a protein molecule free of other cellular components. One means for obtaining an isolated mammalian 5-HT_4 receptor protein is to express DNA encoding the 5-HT_4 receptor in a suitable host, such as a bacterial, yeast, insect, or mammalian cell, using methods well known to those skilled in the art, and recovering the receptor protein after it has been expressed in such a host, again using methods well known in the art. The receptor may also be isolated from cells which express it, in particular from cells which have been transfected with the expression vectors described below in more detail.

This invention provides a vector comprising DNA, RNA, or cDNA, encoding a mammalian 5-HT_4 receptor. This invention further provides a vector comprising DNA, RNA, or cDNA, encoding a human 5-HT_4 receptor. Examples of vectors are viruses such as bacteriophages (such as phage lambda), cosmids, plasmids (such as pUC18, available from Pharmacia, Piscataway, NJ), and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known to those skilled in the art. Examples of such plasmids are plasmids comprising DNA having a coding sequence substantially the same as the coding sequence shown in Figures 1, 2 and 5 (SEQ ID NOs. 1, 3 and 5) and designated pcEXV-S10-87 (ATCC Accession No. 75390), pcEXV-S10-95 (ATCC Accession No. 75391) and pBLuescript-hS10 (ATCC No. 75392).

Alternatively, to obtain these vectors, insert and vector DNA can both be exposed to a restriction enzyme to create complementary ends on both molecules which base pair with each other and are then ligated together with a ligase. Alternatively, linkers can be ligat d to the insert DNA which correspond to a restricti n

site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other means are also available.

This invention also provides vectors comprising a DNA or cDNA encoding a mammalian 5-HT_4 receptor and vectors comprising a DNA or cDNA encoding a human 5-HT_4 receptor, adapted for expression in a bacterial cell, a yeast cell, insect cell or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA or cDNA encoding a mammalian 5-HT_4 receptor or the DNA or cDNA encoding a human 5-HT_4 receptor in the bacterial, yeast, insect or mammalian cells operatively linked to the DNA or cDNA encoding the 5-HT_4 receptor as to permit expression thereof. DNA or cDNA having coding sequence substantially the same as the coding sequence shown in Figures 1 and 2 (SEQ ID NOs. 1 and 3) may be usefully inserted into these vectors to express a mammalian 5-HT_4 receptor. DNA or cDNA having a coding sequence substantially the same as the coding sequence shown in Figure 5 (SEQ ID NO. 5) may be usefully inserted into these vectors to express the human 5-HT_4 receptor. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Maniatis, et al., 1982). Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Furthermore, an insect expression vector, such as recombinant Baculovirus, uses the polyhedrin gene expression signals for expression of the inserted gene

in insect cells. Such vect rs may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express receptors. Certain uses for such cells are described in more detail below.

In one embodiment of this invention a plasmid is adapted for expression in a bacterial, yeast, insect, or, in particular, a mammalian cell wherein the plasmid comprises DNA or cDNA encoding a mammalian 5-HT_4 receptor or DNA or cDNA encoding a human 5-HT_4 receptor and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, insect, or mammalian cell operatively linked to the DNA or cDNA encoding a mammalian 5-HT_4 receptor or to the DNA or cDNA encoding a human 5-HT_4 receptor as to permit expression thereof. Suitable plasmids may include, but are not limited to plasmids adapted for expression in a mammalian cell, e.g., EVJB, EXV-3. An example of such a plasmid adapted for expression in a mammalian cell is a plasmid comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figures 1, 2 and 5 (SEQ ID NOs. 1, 3 and 5) and the regulatory elements necessary for expression of the DNA in the mammalian cell. These plasmids have been designated pcEXV-S10-87 deposited under ATCC Accession No. 75390, pcEXV-S10-95 deposited under ATCC Accession No. 75391, and pBluescript-hS10, deposited under ATCC Accession No 75392. Those skilled in the art will readily appreciate that numerous plasmids adapted for expression in a mammalian cell which comprise DNA encoding a mammalian or human 5-HT_4 receptor and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to contain

th regulatory elements necessary to express the DNA in the mammalian cell. The plasmids may be constructed by th meth ds described above for expression vectors and vectors in general, and by other methods well known in the art.

Deposit discussed supra were made pursuant to, and in satisfaction of, the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

This invention provides a mammalian cell comprising a DNA or cDNA molecule encoding a mammalian 5-HT_4 receptor, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, said plasmid further comprises DNA or cDNA encoding a mammalian 5-HT_4 receptor and the regulatory elements necessary for expression of the DNA or cDNA in the mammalian cell operatively linked to the DNA or cDNA encoding a mammalian 5-HT_4 receptor as to permit expression thereof. This invention provides a mammalian cell comprising a DNA or cDNA molecule encoding a human 5-HT_4 receptor, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, said plasmid further comprises a DNA or cDNA molecule encoding a human 5-HT_4 receptor and the regulatory elements necessary for expression of the DNA or cDNA in the mammalian cell operatively linked to the DNA or cDNA encoding a human 5-HT_4 receptor as to permit expression thereof. Numerous mammalian cells may be used as hosts, including, but not limited to, the mouse fibroblast cell NIH3T3, CHO cells, HeLa cells, LM (tk-) cells, Cos-7 cells, etc. Expression plasmids such as that described supra may be used to transfect mammalian cells by methods well known in the art such as calcium

phosphate precipitation, or DNA or cDNA encoding a human or mammalian 5-HT_4 receptor may be otherwise intr duced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding a human or mammalian 5-HT_4 receptor.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with an unique sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT_4 receptor, for example with a coding sequence included within the sequences shown in Figure 5 (SEQ ID NO. 5). This invention further provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian 5-HT_4 receptor, for example with a coding sequence included within the sequences shown in Figure 1 and Figure 2 (SEQ ID NOs. 1 and 3) As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. As used herein, the phrase "unique sequence" means a nucleic acid molecule sequence specific to only the nucleic acid molecule encoding a mammalian 5-HT_4 receptor. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the pr be. Detection of nucleic acid encoding a human 5-HT_4 receptor is useful as a diagnostic test for any disease process in which levels of expression of th 5-

HT_4 receptor are altered. DNA probe molecules are produced by insertion of a DNA molecule which encodes a 5-HT_4 receptor or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the DNA probes, all using methods well known in the art. For example, the DNA may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the DNA into the vector (discussed above), electrophoresed, and cut out of the resulting gel. An example of such DNA molecules is shown in Figures 1, 2 and 5 (SEQ ID NOs. 1, 3, and 5)> The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA molecule which encode a mammalian 5-HT_4 receptor or complementary to the sequence of a DNA molecule which encodes a human 5-HT_4 receptor are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by the use of amplification techniques such as the polymerase chain reaction.

This invention also provides a method of detecting expression of a human 5-HT_4 receptor on the surface of a cell by detecting the presence of mRNA coding for a 5-HT_4 receptor. This invention further provides a method of detecting expression of a mammalian 5-HT_4 receptor on the surface of the cell by detecting the presence of mRNA coding for a mammalian 5-HT_4 receptor. These methods comprise obtaining total mRNA from the cell using methods well known in the art and contacting

the mRNA so obtained with a nucleic acid probe as described hereinabove, under hybridizing conditi ns, detecting the pr sence of mRNA hybridized to the probe, and thereby detecting the expression of the receptor by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well known in the art. However, in one embodiment of this invention, nucleic acids are extracted by precipitation from lysed cells and the mRNA is isolated from the extract using a column which binds the poly-A tails of the mRNA molecules (Maniatis et al., 1982). The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding may be detected by autoradiography or scintillation counting. However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human 5-HT_4 receptor so as to prevent translation of the human 5-HT_4 receptor. The antisense oligonucleotide may have a sequence capable of binding specifically with any sequences of the cDNA molecule whose sequence is shown in Figure 5 (SEQ ID NO. 5). This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a mammalian 5-HT_4 receptor so as to prevent translation of the mammalian 5-HT_4 receptor. The antisense oligonucleotide may have a sequence capable of binding specifically with any sequences of the cDNA molecule whose sequence is shown in Figures 1 and 2 (SEQ ID NOs. 1 and 3). As used herein, the phrase "binding specifically" means the

ability of an antisense ligonucleotide to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. A particular example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogues of nucleotides.

This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a human 5-HT_4 receptor by passing through a cell membrane and binding specifically with mRNA encoding the 5-HT_4 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. This invention further provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a mammalian 5-HT_4 receptor by passing through a cell membrane and binding specifically with mRNA encoding a mammalian 5-HT_4 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The oligonucleotide may be coupled to a substance which inactivates mRNA, such as a ribozyme. The pharmaceutically acceptable hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a transporter specific for a selected cell type and is thereby taken up by cells of the selected cell type. The structure may be part of

a protein known to bind a cell-type specific transporter, for example an insulin molecule, which would target pancreatic cells. DNA molecules having a coding sequence substantially the same as the coding sequences shown in Figure 5 (SEQ ID No. 5) may be used as the oligonucleotides of the pharmaceutical composition.

This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a mammalian 5-HT_4 receptor by passing through a cell membrane and binding specifically with mRNA encoding the 5-HT_4 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. DNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1 and 2 (SEQ ID NOs. 1 and 3) may be used as the oligonucleotides of the pharmaceutical composition.

This invention provides a method of treating abnormalities which are alleviated by reduction of expression of 5-HT_4 receptor. This method comprises administering to a subject an effective amount of the pharmaceutical composition described above effective to reduce expression of the 5-HT_4 receptor by the subject. This invention further provides a method of treating an abnormal condition related to 5-HT_4 receptor activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to reduce expression of the 5-HT_4 receptor by the subject. Examples of such abnormal conditions are irritable bowel disease, postoperative ileus, diabetic gastroparesis, emesis, achalasia, hiatal hernia, esophageal spasm and other dis ases of the

gastrointestinal tract, as well as in cardiac, urinary, and endocrine function.

Antisense oligonucleotide drugs inhibit translation of mRNA encoding 5-HT_4 receptor. Synthetic antisense oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding the 5-HT_4 receptor and inhibit translation of mRNA and are useful as drugs to inhibit expression of 5-HT_4 receptor genes in patients. This invention provides a means to therapeutically alter levels of expression of a human or mammalian 5-HT_4 receptor by the use of a synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding the 5-HT_4 receptor. Synthetic antisense oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the nucleotide sequence shown in Figure 1, 2 and 5 (SEQ ID NOs. 1, 3 and 5) of DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory cell culture conditions, for administration to cells removed from the patient. The SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable of passing through cell membranes (e.g., by designing small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain selected cell populations by targeting the SAOD to be recognized by specific cellular uptake mechanisms which bind and take up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to

bind to transporter found nly in a certain cell type, as discussed abov . The SAOD is also designed to recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequence shown in Figure 1, 2 and 5 (SEQ ID NOs. 1, 3, and 5) by virtue of complementary base pairing to the mRNA. Finally, the SAOD is designed to inactivate the target mRNA sequence by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular mechanisms such as RNAse I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA targets (Cohen, J.S., 1989; Weintraub, H.M., 1990). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target mRNA (N. Sarver et al., 1990). An SAOD serves as an effective therapeutic agent if it is designed to be administered to a patient by injection, or if the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce 5-HT_4 receptor expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of 5-HT_4 receptor.

This invention provides an antibody directed to the human 5-HT_4 receptor. This invention also provides an antibody directed to the mammalian 5-HT_4 recept r. This antibody may comprise, for example, a monoclonal

antibody directed to an epitope of a human 5-HT_4 receptor present on the surface of a cell, the epitope having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human 5-HT_4 receptor included in the amino acid sequence shown in Figure 5. Amino acid sequences may be analyzed by methods well known to those skilled in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to form the part of the protein that is inserted into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figure 5 will bind to a surface epitope of a 5-HT_4 receptor as described. Antibodies directed to a human or mammalian 5-HT_4 receptor may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as NIH3T3 cells or LM (tk^-) cells may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequence shown in Figures 1, 2, and 5 (SEQ ID NOs. 1-6). As a still further alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of 5-HT_4 receptor encoded by the isolated DNA, or to inhibit the function of the 5-HT_4 receptor in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of the human 5-HT_4 receptor, effective to block binding of naturally occurring substrates to the 5-HT_4 receptor, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a human 5-HT_4 receptor present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human 5-HT_4 receptor included in the amino acid sequence shown in Figure 5 (SEQ ID NOs. 5 and 6) is useful for this purpose.

This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of a mammalian 5-HT_4 receptor, effective to block binding of naturally occurring substrates to the 5-HT_4 receptor, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a mammalian 5-HT_4 receptor present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of a mammalian 5-HT_4 receptor included in the amino acid sequence shown in Figures 1 and 2 (SEQ ID NOs. 1-4) is useful for this purpose.

This invention also provides a method of treating abnormalities in a subject which are alleviated by reduction of expression of a human or mammalian 5-HT_4 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition described above effective to block binding of naturally occurring substrates to the receptor and thereby alleviate abnormalities resulting from overexpression of a human or mammalian 5-HT_4 receptor. Binding of the antibody to the receptor prevents the receptor from

functioning, thereby neutralizing th effects of over xpression. The monocl nal antibodies described above are useful for this purpose. This invention additionally provides a method of treating an abnormal condition related to an excess of 5-HT_4 receptor activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring substrates to the 5-HT_4 receptor and thereby alleviate the abnormal condition. Some examples of abnormal conditions associated with excess 5-HT_4 receptor activity are irritable bowel disease, postoperative ileus, diabetic gastroparesis, emesis, achalasia, hiatal hernia, esophageal spasm and other diseases of the gastrointestinal tract, as well as in cardiac, urinary, and endocrine function.

This invention provides methods of detecting the presence of a 5-HT_4 receptor on the surface of a cell which comprises contacting the cell with an antibody directed to the 5-HT_4 receptor, under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby the presence of the 5-HT_4 receptor on the surface of the cell. Such methods are useful for determining whether a given cell is defective in expression of 5-HT_4 receptors. Bound antibodies are detected by methods well known in the art, for example by binding fluorescent markers to the antibodies and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

This invention provides a transgenic nonhuman mammal expressing DNA enc ding a human 5-HT_4 receptor and a transgenic nonhuman mammal expressing DNA encoding a

mammalian 5-HT_4 recept r. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a human or mammalian 5-HT_4 receptor so mutated as to be incapable of normal receptor activity, and not expressing native 5-HT_4 receptor. This invention further provides a transgenic nonhuman mammal whose genome comprises DNA encoding a human 5-HT_4 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a human 5-HT_4 receptor and which hybridizes to mRNA encoding a 5-HT_4 receptor thereby reducing its translation and a transgenic nonhuman mammal whose genome comprises DNA encoding a mammalian 5-HT_4 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a mammalian 5-HT_4 receptor and which hybridizes to mRNA encoding a mammalian 5-HT_4 receptor thereby reducing its translation. The DNA may additionally comprise an inducible promoter or additionally comprise tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell types. Examples of DNA are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figure 1,2 and 5 (SEQ ID NOs. 1, 3, and 5). An example of a transgenic animal is a transgenic mouse. Examples of tissue specificity-determining regions are the metallothionein promotor (Low et al., 1986) and the L7 promotor (Oberdick et al., 1990).

Animal model systems which elucidate the physiological and behavioral roles of mammalian receptors are produced by creating transgenic animals in which the expression of a receptor is either increased or decreased, or the amino acid sequence of the expressed recept r protein is altered, by a variety of techniques. Examples of these techniques include, but ar not limited to: 1) Insertion of normal or mutant

versions of DNA encoding a human 5-HT_4 receptor or homologous animal versions of this gene, by microinjection, retroviral infecti n or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal (Hogan et al., 1986) or, 2) Homologous recombination (Capecchi M.R., 1989; Zimmer A, and Gruss, P., 1989) of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of the receptor. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native receptor but does express, for example, an inserted mutant receptor, which has replaced the native receptor in the animal's genome by recombination, resulting in underexpression of the receptor. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added receptors, resulting in overexpression of the receptor.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium (Hogan, B. et al. 1986). DNA or cDNA encoding a receptor is purified from a vector (such as plasmids pcEXV-S10-87, pcEXV-S10-95 and pBluescript-hS10 described above) by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the c ding region to permit tissue-specific expression of

the trans-gene. The DNA, in an appropriately buffered solution, is put int a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

Since the normal action of receptor-specific drugs is to activate or to inhibit the receptor, the transgenic animal model systems described above are useful for testing the biological activity of drugs directed against the receptors even before such drugs become available. These animal model systems are useful for predicting or evaluating possible therapeutic applications of drugs which activate or inhibit receptors by inducing or inhibiting expression of the native or trans-gene and thus increasing or decreasing expression of normal or mutant receptors in the living animal. Thus, a model system is produced in which the biological activity of drugs directed against the receptors are evaluated before such drugs become available. The transgenic animals which over or under produce the receptor indicate by their physiological state whether over or under production of the receptor is therapeutically useful.

It is therefore useful to evaluate drug action based on the transgenic model system. One use is based on the fact that it is well known in the art that a drug such as an antidepressant acts by blocking neurotransmitter

uptake, and ther by increases the amount of neur transmitter in the synaptic cleft. The physiol gical result f this action is to stimulat the production of less receptor by the affected cells, leading eventually to underexpression. Therefore, an animal which underexpresses receptor is useful as a test system to investigate whether the actions of such drugs which result in under expression are in fact therapeutic. Another use is that if overexpression is found to lead to abnormalities, then a drug which down-regulates or acts as an antagonist to the receptor is indicated as worth developing, and if a promising therapeutic application is uncovered by these animal model systems, activation or inhibition of the 5-HT_4 receptor is achieved therapeutically either by producing agonist or antagonist drugs directed against the 5-HT_4 receptor or by any method which increases or decreases the expression of this receptor in man.

Further provided by this invention is a method of determining the physiological effects of expressing varying levels of human or mammalian 5-HT_4 receptors which comprises producing a transgenic nonhuman animal whose levels of human or mammalian 5-HT_4 receptor expression are varied by use of an inducible promoter which regulates receptor expression. This invention also provides a method of determining the physiological effects of expressing varying levels of human or mammalian 5-HT_4 receptor which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human or mammalian 5-HT_4 receptor. Such animals may be produced by introducing different amounts of DNA encoding a human or mammalian 5-HT_4 receptor into the oocytes from which the transgenic animals are developed.

This invention also provides a method for identifying

a substance capable of alleviating abnormalities resulting fr m overexpression of a human or mammalian 5-HT_4 receptor comprising administering the substance to a transgenic nonhuman mammal expressing at least one artificially introduced DNA molecule encoding a human or mammalian 5-HT_4 receptor and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a human or mammalian 5-HT_4 receptor. As used herein, the term "substance" means a compound or composition which may be natural, synthetic, or a product derived from screening. Examples of DNA molecules are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1, 2, and 5 (SEQ ID NOs. 1, 3, and 5).

This invention provides a pharmaceutical composition comprising an amount of the substance described supra effective to alleviate the abnormalities resulting from overexpression of 5-HT_4 receptor and a pharmaceutically acceptable carrier.

This invention further provides a method for treating the abnormalities resulting from overexpression of a human or mammalian 5-HT_4 receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from overexpression of a human or mammalian 5-HT_4 receptor.

This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human or mammalian 5-HT_4 receptor comprising administering the substance to the transgenic nonhuman mammal described above which expresses only nonfunctional human or mammalian 5-HT_4

receptor and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a human or mammalian 5-HT_4 receptor.

This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a human or mammalian 5-HT_4 receptor and a pharmaceutically acceptable carrier.

This invention further provides a method for treating the abnormalities resulting from underexpression of a human or mammalian 5-HT_4 receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a human or mammalian 5-HT_4 receptor.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a human or mammalian 5-HT_4 receptor allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human or mammalian 5-HT_4 receptor and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a human or mammalian 5-HT_4 receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and

g) comparing the unique band pattern specific to the DNA of subj cts suffering from the disorder from step e and th DNA obtained for diagnosis from st p f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human 5-HT_4 receptor allele or mammalian 5-HT_4 receptor allele.

This invention provides a method of preparing the isolated 5-HT_4 receptor which comprises inducing cells to express receptor, recovering the receptor from the resulting cells, and purifying the receptor so recovered. An example of a 5-HT_4 receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 5. For example, cells can be induced to express receptors by exposure to substances such as hormones. The cells can then be homogenized and the receptor isolated from the homogenate using an affinity column comprising, for example serotonin or another substance which is known to bind to the 5-HT_4 receptor. The resulting fractions can then be purified by contacting them with an ion exchange column, and determining which fraction contains 5-HT_4 receptor activity or binds anti-receptor antibodies.

This invention provides a method of preparing an isolated human 5-HT_4 receptor which comprises inserting nucleic acid encoding the human 5-HT_4 receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered. An example of an isolated human 5-HT_4 receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence

sh wn in Figure 5 (SEQ ID NOs. 5 and 6). This invention provides a m thod f preparing an isolated mammalian 5-HT_4 receptor which compris s inserting nucleic acid encoding the mammalian 5-HT_4 receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered. An example of an isolated mammalian 5-HT_4 receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figures 1 and 2 (SEQ ID NOs. 1- 2 and Seq I. D. Nos. 3-4, respectively). These methods for preparing 5-HT_4 receptor uses recombinant DNA technology methods well known in the art. For example, isolated nucleic acid encoding 5-HT_4 receptor is inserted in a suitable vector, such as an expression vector. A suitable host cell, such as a bacterial cell, insect cell, or a eukaryotic cell such as a yeast cell, is transfected with the vector. 5-HT_4 receptor is isolated from the culture medium by affinity purification or by chromotography or other methods well known in the art.

This invention provides a method for determining whether a compound not known to be capable of specifically binding to a human 5-HT_4 receptor can specifically bind to the human 5-HT_4 receptor, which comprises contacting a mammalian cell comprising a plasmid adapted for expression in a mammalian cell which plasmid further comprises a DNA which expresses a human 5-HT_4 receptor on the cell's surface with the compound under conditions permitting binding of ligands known to bind to a human 5-HT_4 receptor, detecting the presence of any compound bound to the human 5-HT_4 receptor, the presence of bound compound indicating that the compound is capable of specifically binding to the human 5-HT_4 receptor.

This invention provides a method for determining wh ther a compound not known to be capable of specifically binding t a mammalian 5-HT_4 receptor can specifically bind to the mammalian 5-HT_4 receptor, which comprises contacting a mammalian cell comprising a plasmid adapted for expression in a mammalian cell which plasmid further comprises a DNA which expresses a mammalian 5-HT_4 receptor on the cell's surface with the compound under conditions permitting binding of ligands known to bind to a mammalian 5-HT_4 receptor, detecting the presence of any compound bound to the human 5-HT_4 receptor, the presence of bound compound indicating that the compound is capable of specifically binding to the mammalian 5-HT_4 receptor.

This invention provides a method for identifying a compound which is not known to be capable of binding to a human 5-HT_4 receptor can functionally activate the human 5-HT_4 receptor on the surface of a mammalian cell or prevent a ligand which does so, which comprises contacting the mammalian cell which cell comprises a plasmid adapted for expression in the mammalian cell such plasmid further comprising DNA which expresses the human 5-HT_4 receptor on the surface of the mammalian cell with the compound, determining whether the compound activates the human 5-HT_4 receptor or prevents a ligand which does so, and thereby identifying the compound as a compound which is binds to and functionally activates the human 5-HT_4 receptor or prevents the functional activation of the human 5-HT_4 receptor by a ligand which does so.

The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 5 (SEQ ID No. 5).

This invention provides a method for identifying a compound which is not known to be capabl of binding to

a mammalian 5-HT_4 receptor can functi nally activate the mammalian 5-HT_4 receptor on the surface of a mammalian cell or prevent a ligand which does so, which comprises contacting the mammalian cell which cell comprises a plasmid adapted for expression in the mammalian cell such plasmid further comprising DNA which expresses the mammalian 5-HT_4 receptor on the surface of the mammalian cell with the compound, determining whether the compound activates the mammalian 5-HT_4 receptor or prevents a ligand which does so, and thereby identifying the compound as a compound which is binds to and functionally activates the mammalian 5-HT_4 receptor or prevents the functional activation of the mammalian 5-HT_4 receptor by a ligand which does so. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figures 1 and 2 (SEQ ID NOs. 1 and 3).

The activation or blockade of the functional response is detected by means of a bioassay from the mammalian cell such as a second messenger response, and thereby determining whether the compound activates or prevents the activation of the 5-HT_4 receptor functional output. Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is an LM (tk-) cell. Another example of a non-neuronal mammalian cell to be used for functional assays is a murine fibroblast cell line, specifically the NIH3T3 cell. The preferred method for determining whether a compound is capable of binding to the 5-HT_4 receptor comprises contacting a transfected nonneuronal mammalian cell (i.e. a cell that does not naturally express any type of 5-HT or G-protein coupled receptor, thus will only express such a receptor if it is transfected into the cell) expressing a 5-HT_4 receptor on its surface, or contacting a membrane preparation derived from such a transfected cell, with the compound

under conditions which are known to prevail, and thus to be associated with, in vivo binding of ligands to a 5-HT_4 recept r, detecting the pr sence of any of the compound being tested bound to the 5-HT_4 receptor on the surface of the cell, and thereby determining whether the compound binds to, and activates or prevents the activation of the 5-HT_4 receptor. This response system is obtained by transfection of isolated DNA into a suitable host cell containing the desired second messenger system such as phosphoinositide hydrolysis, adenylate cyclase, guanylate cyclase or ion channels. Such a host system is isolated from pre-existing cell lines, or can be generated by inserting appropriate components of second messenger systems into existing cell lines. Such a transfection system provides a complete response system for investigation or assay of the activity of human 5-HT_4 receptor with compounds as described above.

Transfection systems are useful as living cell cultures for competitive binding assays between known or candidate drugs and ligands which bind to the receptor and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the receptor isolated from transfected cells are also useful for these competitive binding assays. Functional assays of second messenger systems or their sequelae in transfection systems act as assays for binding affinity and efficacy in the activation of receptor function. A transfection system constitutes a "drug discovery system" useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate or inhibit the natural functions of the human 5-HT_4 receptor. The transfection system is also useful for determining the affinity and efficacy of known drugs at

human 5-HT_4 receptor sites.

This invention also provides a method of screening compounds to identify drugs which interact with, and specifically bind to, a human 5-HT_4 receptor on the surface of a cell, which comprises contacting a mammalian cell which comprises a plasmid adapted for expression in a mammalian cell which plasmid further comprises DNA which expresses a human 5-HT_4 receptor on the cell's surface with a plurality of compounds, determining those compounds which bind to the human 5-HT_4 receptor expressed on the cell surface of the mammalian cell, and thereby identifying compounds which interact with, and specifically bind to, the human 5-HT_4 receptor. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 5 (SEQ ID NO. 5). This invention also provides a method of screening compounds to identify drugs which interact with, and specifically bind to, a mammalian 5-HT_4 receptor on the surface of a cell, which comprises contacting a mammalian cell which comprises a plasmid adapted for expression in a mammalian cell which plasmid further comprises DNA which expresses a mammalian 5-HT_4 receptor on the cell's surface with a plurality of compounds, determining those compounds which bind to the mammalian 5-HT_4 receptor expressed on the cell surface of the mammalian cell, and thereby identifying compounds which interact with, and specifically bind to, the mammalian 5-HT_4 receptor. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figures 1 and 3 (SEQ ID NOs. 1 and 2). Various methods of detection may be employed. The compounds may be "labeled" by association with a detectable marker substance (e.g., radiolabel or a non-is topic label such as biotin). Preferably, the mammalian cell is nonneuronal in origin. An example of

a nonneuronal mammalian cell is a Cos-7 cell. Drug candidates are identified by choosing chemical compounds which bind with high affinity to the expressed 5-HT_4 receptor protein in transfected cells, using radioligand binding methods well known in the art, examples of which are shown in the binding assays described herein. Drug candidates are also screened for selectivity by identifying compounds which bind with high affinity to one particular receptor but do not bind with high affinity to any other receptor subtypes or to any other known receptor. Because selective, high affinity compounds interact primarily with the target 5-HT_4 receptor site after administration to the patient, the chances of producing a drug with unwanted side effects are minimized by this approach.

This invention provides a pharmaceutical composition comprising a drug identified by the method described above and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. Once the candidate drug has been shown to be adequately bioavailable following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bioavailable, in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

Applicants have identified a novel 5-HT receptor subtype protein, designated 5-HT_4 and have described methods for the identification of pharmacological compounds for therapeutic treatments. Pharmacological compounds which are directed against specific receptor subtypes provide effective new therapies with minimal side effects.

Elucidation of the molecular structures of the neuronal serotonin receptors is an important step in the understanding of serotonergic neurotransmission. This disclosure reports the isolation and amino acid sequence of a novel cDNA which encodes a human 5-HT_4 receptor. This disclosure reports the isolation, amino acid sequence, and functional expression of a two novel cDNAs which encode mammalian 5-HT_4 receptors. The identification of 5-HT receptor subtypes play a pivotal role in elucidating the molecular mechanisms underlying serotonergic transmission, and should also aid in the development of novel therapeutic agents.

A complementary DNA clone (designated pBluescript-hS10) encoding a serotonin receptor subtype, 5-HT_4, has been isolated from human brain, human heart and human retina. Additionally, two complementary DNA clones encoding the serotonin 5-HT_4 receptor subtype have been isolated from mammalian brain and their functional properties have been examined in mammalian cells. Analysis of 5-HT_4 structure and function provides a model for the development of drugs useful for the treatment of gastrointestinal conditions including irritable bowel disease, postoperative ileus, diabetic gastroparesis, emesis, achalasia, hiatal hernia, and esophageal spasm. In addition, 5-HT_4 receptors have been described functionally in the heart (Kaumann,

1992), adrenal (Lefebvre et al., 1992), and bladder (Corsi et al., 1991) indicating p ssible roles in cardiac rate and force of contraction, end crine control of cortisol secretion, and urinary incontinence or spasticity. 5-HT_4 receptors have also been described in the brain, particularly in areas such as the hippocampus, in which we have localized the gene encoding 5-HT_4 receptors (S10-95), indicating a potential role in cognitive enhancement (Bockaert et al., 1992).

This invention identifies a mammalian serotonin receptor, its amino acid sequence, and its mammalian gene, the activation of which is coupled to activation of adenylate cyclase. The information and experimental tools provided by this discovery are useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this receptor protein, its associated mRNA molecule or its associated genomic DNA. The information and experimental tools provided by this discovery will be useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new serotonin receptor subtype, its associated mRNA molecule, or its associated genomic DNA.

Specifically, this invention relates to the isolation of human cDNA clone and mammalian cDNA clones encoding a new serotonin receptor, designated 5-HT_4. In addition, the mammalian 5-HT_4 receptors have been expressed in COS-7 cells by transfecting the cells with the plasmids pcEXV-S10-87 and pcEXV-S10-95. The pharmacological binding properties of the encoded 5-HT_4 receptor have been determined, and the binding properties classify this receptor as a novel serotonin receptor. Mammalian cell lines expressing the mammalian 5-HT_4 receptor on the cell surface have been constructed, thus establishing the first well-defined,

cultured cell lines with which to study the novel 5-HT_4 receptor.

The invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative, and are not meant to limit the invention as described herein, which is defined by the claims which follow thereafter.

Methods and Materials

PCR Amplification: The third (III) and fifth (V) transmembrane (TM) domains of the following receptors were aligned and used to synthesize a pair of degenerate primers: 5-HT_{1A}, 5-HT_{1C}, 5-HT_2 and the 5-$HT_{1D\alpha/\beta}$ receptors. Primers 3.17 and 5.5 ([5'-TGGAATTCTG(C/T)G(C/T)IAT(A/C/T)(G/T)CICTGGA(C/T)(A/C)G(C/G)TA-3'] (SEQ ID No. 9), [5'-CATIA(G/C/A)I(G/A)IIA(G/A)IGG(T/G/A/)AT(G/A)(T/A)A(G/A)AAIGC-3']) (SEQ ID No. 10) were used to amplify 5 μg of poly (A+) RNA from rat brain that was reverse transcribed by avian myeloblastosis virus reverse transcriptase (AMV). PCR was performed on single-stranded cDNA under the following conditions: 94°C for 1 min, 50°C for 2 min and 72°C for 3 min for 40 cycles. Following PCR, 90 μl of the reaction was phenol:chloroform extracted and precipitated; 10 μl was visualized on a gel using ethidium bromide staining. After precipitation the sample was treated with T4 DNA polymerase and digested with EcoR1 prior to separation on a 1% agarose gel. The DNA fragments (200 to 400 base pairs) were isolated from the gel, kinased and cloned into pBluescript. Recombinant clones were analyzed by sequencing. One fragment 270 base pairs in length, named S10, was identified. This sequence contained a "TM IV" like domain and represented a potentially new serotonin receptor. The corresponding full length cDNA was isolated from a rat brain cDNA library.

Rat PCR primers (from TM3 to TM7) were used to amplify single-stranded cDNA prepared from human heart, brain and retina, as described above. Those human PCR DNA fragments were subcloned in pBluescript and sequenced.

cDNA Library Constructi n, Screening and Sequencing:

Rat brains were dissected fr m adult male CD rats (Charles River Laboratories) and total RNA was prepared by the guanidine thiocyanate method (Chirgwin, J.W. et al.; 1979). Poly A$^+$RNA was purified with a Fast track kit (Invitrogen Corp., San Diego, CA). Double stranded (DS) cDNA was synthesized from 5 μg of poly A$^+$RNA according to Gubler and Hoffman (Gubler, U. and B. J. Hoffman, 1983). The resulting DS cDNA was ligated to BstxI/EcoRI adaptors (Invitrogen Corp.), the excess of adaptors was removed by chromatography on Sepharose CL 4B (Pharmacia LKB) and the DNA was then size selected on a Gen-Pak Fax HPLC column (Zhao, D. et al., 1992) (Waters, Millipore Corp., Milford, MA). High molecular weight fractions were ligated in pCDM8 cut by BstxI (Invitrogen Corp.). The ligated DNA was electroporated in E.Coli MC 1061 (Gene Pulser, Biorad). A total of 20 x 10^6 independent clones with an insert mean size of 1.9 kb could be generated. Before amplification, the library was divided into pools of 2.5 to 5 x 10^4 independent clones. After 18 hours amplification, the pools were stored at -85°c in 20% glycerol.

100 pools of the cDNA library, representing 3.2 x 10^6 primary clones, were screened using exact PCR primers derived from the S10 PCR clone sequence. 1 μl (4 x 10^6 bacteria) of each amplified pool was subjected directly to 40 cycles of PCR and the resulting products analyzed by agarose gel electrophoresis and Southern blotting. Two out of four positive pools were analyzed further and by sib selection and plating out, two individual full length cDNA clones, S10-87 and S10-95, were isolated. DS-DNA was sequenced with a sequanase kit (US Biochemical, Cleveland, OH) according to the manufacturer. Nucleotide and peptide sequences analysis were performed with GCG programs.

Genomic Cloning and S quencing: A human fibroblast

genomic library in λ dash II (≈1.5 x 10^6 total recombinants; Stratagene, LaJolla, CA) was screened using a 45 nt. oligonucleotide probe derived from the rat S10-87 receptor gene, designed in the 3′ end of the carboxyl terminal tail (from the anti-sense strand [nucleotide 1220-1264), 5′ TCAAAAGCATGATTCCAGGGACTCTGGGTCATTGTGTATGGG CAA 3′ (SEQ ID No. 11) (see Fig.1). The oligomer was labeled with [^{32}P]γATP by using polynucleotide kinase. Hybridization was performed at medium stringency conditions: 45°C. in a solution containing 37.5% formamide, 5x SSC (1X SSC is 0.15M sodium chloride, 0.015M sodium citrate), 1x Denhardt's solution (0.02% polyvinylpyrrolindone, 0.02% Ficoll, 0.02% bovine serum albumin), and 200 μg/μl sonicated salmon sperm DNA. The filters were washed at 45°C. in 0.1x SSC containing 0.1% sodium dodecyl sulfate and exposed at -70°C. to Kodak XAR film in the presence of an intensifying screen. Lambda phage clones hybridizing with the probe were plaque purified and DNA was prepared for Southern blot analysis (Southern, 1975; Sambrook et al., 1989). A 900 bp Hind2/SstI hybridizing fragment was subcloned into pUC18 (Pharmacia, Piscataway, NJ)). Nucleotide sequence analysis was accomplished by the Sanger dideoxy nucleotide chain termination method (Sanger et al., 1977) on denatured double-stranded plasmid templates, using Sequenase (US Biochemical Corp., Cleveland, OH).

PCR amplification of a partial length human S10-87 cDNA clone:

The 900 bp Hind2/SstI fragment contained sequence encoding the human S10-87 carboxy terminal tail, including the stop codon. This sequence was used to generate a 25 mer (reverse primer) containing the stop codon: 5′ CCTCAATCAGAAGCATGATTCCAGG 3′(SEQ ID No. 12). As a forward primer we used the 5′ end f the human PCR

fragment previously identified (figure 6): 5'TTGGTCTATAGGAACAAGATGACCC 3' (SEQ ID No. 13). These human PCR primers w re used to amplify single stranded cDNA prepared from human brain as previously described. The amplified DNA was subcloned and sequenced as described above.

Isolation of the full length human S10-95 cDNA clone: 20 pools of a human hippocampal cDNA library (3kb average size insert, in pcEXV-3) representing 10^6 independent clones were screened by PCR with TM4-TM6 primers as previously described. Five positive pools were identified. one of those pools was analyzed further and by sib selection a 5kb cDNA clone, CG-17, was isolated. Double Stranded-DNA was sequenced as described above. Nucleotide and peptide sequence analysis were performed with the Genetics Computer Group sequence analysis software package.

DNA transfection: The full coding region of S10-87 (clone CG-5) and S10-95 (clones CG-6 and CG-17) were subcloned in the correct orientation in the mammalian expression vectors pCDNA1-Amp (Invitrogen Corp.), and pcEXV-3 (Miller, J. and R. N. Germain, 1986) (CG-7 and CG-8 respectively). For transient expression, Cos-7 cells were transfected by the DEAE-Dextran method, using 1 μg of DNA /10^6 cells (Warden, D. and H.V. Thorne, 1968).

Membrane Preparation: Membranes were prepared from transiently transfected COS-7 cells which were grown to 100% confluence. The cells were washed twice with phosphate-buffered saline, scraped from the culture dishes into 5 ml of ice-cold phosphate-buffered saline, and centrifuged at 200 x g for 5 min at 4°. The pellet was resuspended in 2.5 ml of ice-cold Tris buffer (20 mM Tris -HCl, pH 7.4 at 23°, 5 mM EDTA), and

homogenized by a Wheaton tissue grinder. The lysate was subsequently centrifuged at 200 x g for 5 min at 4° t pellet large fragments which were discarded. The supernatant was collected and centrifuged at 40,000 x g for 20 min at 4°. The pellet resulting from this centrifugation was washed once in ice-cold Tris wash buffer and finally resuspended in a final buffer containing 50 mM Tris-HCl and 0.5 mM EDTA, pH 7.4 at 23°. Membrane preparations were kept on ice and utilized within two hr for the radioligand binding assays. Protein concentrations were determined by the method of Bradford (1976) using bovine serum albumin as the standard.

Radioligand Binding: [^{3}H]5-HT binding was performed using slight modifications of the 5-HT_{1D} assay conditions reported by Herrick-Davis and Titeler (1989) with the omission of masking ligands. Radioligand binding studies were achieved at 37° C in a total volume of 250 µl of buffer (50 mM Tris, 10 mM $MgCl_2$, 0.2 mM EDTA, 10 µM pargyline, 0.1 % ascorbate, pH 7.4 at 37° C) in 96 well microtiter plates. Saturation studies were conducted using [^{3}H]5-HT at 10 different concentrations ranging from 1.0 nM to 100 nM. Displacement studies were performed using 10 nM [^{3}H]5-HT. The binding profile of drugs in competition experiments was established using 7 concentrations of compound. Incubation times were 30 min for both saturation and displacement studies. Nonspecific binding was defined in the presence of 10 µM 5-HT. Binding was initiated by the addition of 50 µl membrane homogenates (10-20 µg). The reaction was terminated by rapid filtration through presoaked (0.5% polyethyleneimine) filters using 48R Cell Brandel Harvester (Gaithersburg, MD). Subsequently, filters were washed for 5 sec with ice cold buffer (50 mM Tris HCL, pH 7.4 at 4° C), dried and placed into vials

containing 2.5 ml of Readi-Safe (Beckman, Fullerton, CA), and radioactivity was measured using a Beckman LS 6500C liquid scintillation counter. Th efficiency of counting of [^{3}H]5-HT averaged between 45-50%. Binding data were analyzed by computer-assisted nonlinear regression analysis (Accufit and Accucomp, Lundon Software, Chagrin Falls, OH). IC_{50} values were converted to K_i values using the Cheng-Prusoff equation (1973).

[^{3}H]GR113808 binding was performed using slight modifications of the method of Waeber et al., 1993. Radioligand binding studies were achieved at 37°C in a total volume of 250 µl of buffer (50 mM Tris, 10 µM, 0.01% ascorbate, pH 7.4 at 37°C) in 96 well microtiter plates. Saturation studies were conducted using [^{3}H]GR113808 at 10-12 different concentrations ranging from 0.005-2.5 nM. Displacement studies were performed using 0.2-0.4 nM [^{3}H]GR113808. The binding profile of drugs in competition experiments was established using 10-12 concentrations of compound. Incubation times were 30 min for both saturation and displacement studies. Nonspecific binding was defined in the presence of 50 µM 5-HT. Binding was initiated and terminated as described for [^{3}H]5-HT binding (see above). Radioactivity was measured and data were analyzed as described above for [3H]5-HT.

Measurement of cAMP Formation: The transiently transfected Cos-7 cells were incubated in Dulbecco's modified Eagle's medium, 5 mM theophylline, 10 mM Hepes (4-[2-Hydroxyethyl]-1-piperazineethanesulfonic acid), 10 µM pargyline, and/or appropriate concentrations of antagonists for 20 minutes at 37°C, 5% CO_2. Serotonin or other agonists in the presence or absence of forskolin (FSK) (10 µM) were then added at appropriate concentrations and incubated for an additional 10

minutes at 37°C, 5% CO_2. The media was aspirated and the reaction stopped by the addition of 100 mM HCl. The plates were stored at 4°C for 15 minutes, centrifuged for 5 minutes, 500 x g to pellet cellular debris, and the supernatant aliquotted and stored at -20°C prior to assessment of cAMP formation by radioimmunoassay (cAMP Radioimmunoassay kit, Advanced Magnetics, Cambridge, MA). Radioactivity was quantitated using a Packard COBRA Auto Gamma Counter equipped with data reduction software. Functional data was fitted to a four parameter logistic equation to obtain response parameters (EC_{50}, E_{max}, nH; Inplot, GraphPad, San Diego, CA).

Drugs: [^{3}H]5-HT (specific activity = 22.7 Ci/mmole) was obtained from New England Nuclear, Boston, MA. [^{3}H]GR113808 (specific activity = 82 Ci/mmol) was obtained from Amersham International (Arlington Hts., IL). All other chemicals were obtained from commercial sources and were of the highest grade purity available.

Experimental Results

A 270 bp DNA fragment (S10) was identified when rat brain cDNA was used as template in a PCR amplification with two degenerate oligonucleotide primers derived from well conserved regions among several serotonin receptors, in the third and fifth putative transmembrane domains. The peptide sequence corresponding to this S10 PCR clone contained a "transmembrane IV like" domain. Since we used "serotonin receptor specific" PCR primers, this S10 clone represented a potentially new serotonin receptor. The corresponding full length cDNA was isolated from a rat brain cDNA library. Since five amplified commercial phage cDNA libraries turned out to be negative, we split the plasmid cDNA library into small pools of 2.5 to 5 x 10^4 independent clones before amplification to avoid a potential growth bias against the S10 cDNA clone. By direct PCR analysis of bacterial pools, subsequent sib selection and standard filter hybridization, two cDNA clones were identified, S10-87 (5.5 kb) and S10-95 (4.5 kb). The nucleotide and deduced amino acid sequences are shown in Figure 1 (S10-87) and Figure 2 (S10-95). Surprisingly the peptide sequences between those two clones are only 96.7% identical, diverging in the second half of the carboxy terminus tails, downstream of position 359 (Figure 3). In addition, the entire 3' untranslated regions are totally divergent. The longest open reading frame for S10-87 encodes a protein of 387 amino acids and 406 amino acids for S10-95. The hydrophobicity plot displayed seven hydrophobic, putative membrane spanning regions which when compared to other G protein-coupled receptors did not show any significant high homologies, even to other serotonin receptors (Table 1 and figure 4). It is interesting to not that the highest homology, overall or restricted

to the 7 TM region, is exhibited by the human histamine H_2 receptor, which like the 5-HT_4 rec ptor, is coupled t stimulation of cAMP.

Both S10-87 and S10-95 proteins carry 4 potential N-glycosylation sites in positions 7, 180, 316, and 352. They also possess 3 potential phosphorylation sites for protein kinase C in positions 218, 248, 318 and 4 potential phosphorylation sites for casein kinase II in positions 9, 97, 218 and 288. A potential palmitoylation site is present in both clones at the cysteine found in position 329. A large number of G protein-coupled receptors carry a cysteine in the same position and O'Dowd et al. have speculated that it plays an important role in the functional coupling of the human β_2-adrenergic receptor. In addition, clone S10-95 carries one more potential phosphorylation site for protein kinase C at position 400. This additional phosphorylation site could lead to differential functional coupling between the S10-87 and S10-95 receptors.

Since we isolated two different S10 cDNA clones by screening a library made from an entire brain, we checked for differential expression in seven different parts of the brain by PCR amplification using pairs of primers specific for each clone. The results are summarized in table 2. Clone S10-95 seems to be transcribed everywhere in the rat brain except in cerebellum. Clone S10-87 is only expressed in striatum. It remains to be determined if only one or both receptors are expressed in rat cortex.

The partial human S10-87 nucleotide (Fig 11A) and deduced amino acid sequences (Fig 11B) are shown. The sequences are highly similar to the rat S10-87 homolog, 90.8 % at the nucleotide level and 93.8 % at the amino

acid level (figures 12 and 13 respectively).

Th full length human S10-95 nucl otide (Fig 14A)and deduced amino acid sequences (Fig 14B) are shown. Compared to the rat S10-95 sequence, it shows 90.7 % identity at the nucleotide level and 91.8 % identity at the amino acid level (figures 15 and 16 respectively). The human S10-95 nucleotide sequence contains one nucleotide insertion in position 1159. This insertion creates a frame shift and introduces a stop codon in the reading frame 16 nucleotides downstream. The protein motifs are highly conserved between the rat and human homologs except for a casein kinase II potential phosphorylation site in position 288 which is lost in both human receptors. The human homologs both carry a potential cAMP/cGMP phosphorylation site in position 338 in their carboxy terminal tail which is absent in the rat homologs. A comparison of the amino acid sequence between the human and the rat S10-95 clones beginning from the initiating methionine and ending with the stop codon of the human S10-95 clone, reveals 31 amino acid changes of which 11 are non conservative, including 2 in TM1, 1 in TM2 and 1 in TM4. Due to the nucleotide insertion and the corresponding frame shift described above, the carboxy terminal tail of the human S10-95 receptor is 16 amino acid shorter than its rat homolog.

Identical to the rat homologs, both human clones are identical in the loops and transmembrane regions, differing only in the second half of their carboxy terminal tail (fig 17, nucleic acid sequence; fig 18, aa sequence).

The human PCR cDNA fragments (TM-3 to TM-7) are 100% identical between heart, brain and retina. The nucleotide and deduced amino acid sequences are shown

in figure 5. The human sequence shows 90.7% homology with the rat nucleotide sequence (Figure 6) and 92.3% homology (Figure 7) with the rat amino acid sequence.

The genes encoding the rat S10-87 and S10-95 receptors were transiently expressed in Cos-7 cells for pharmacological evaluation. Initial experiments using 5 nM [^{3}H]5-HT indicated that both S10-87 and S10-95 were serotonergic sites as demonstrated by the degree of specific binding and density of sites expressed in the transfected cells when compared against the mock transfected controls. Saturation analysis of S10-87 (CG-7) was performed using 10 concentrations of [^{3}H]5-HT (1-100 nM) and yielded a Bmax of 1,938 ± 399 fmol/mg of protein and a K_d for [^{3}H]5-HT of 7.87 ± 0.06 nM. The degree of specific binding at concentrations of [^{3}H]5-HT close to the K_d ranged from 70-84% throughout the experimental series (including saturation and competition studies). Although the use of [^{3}H]5-HT as a radioligand to label 5-HT_4 receptors in brain tissue is not efficient due to the nonselectivity of the ligand, it became clear in the present studies using a homogeneous receptor population that [^{3}H]5-HT would label this particular receptor. In fact, [^{3}H]5-HT appears to be labelling the high affinity state of the 5-HT_4 receptor which is not unusual for the conditions upon which this receptor has been studied. Similar results using an agonist radioligand have been previously reported for the cloned 5-HT_2 receptor (Branchek et al., 1990).

A pharmacological binding profile of S10-87 and S10-95 (CG-7 and CG-8) was performed and demonstrated that this novel receptor was similar to the 5-HT_4 receptor as defined by functional assays in the literature (Bockaert et al., 1992). This is clearly shown in table 3 where the binding affinities of various

serotonergic ag nts are displayed for S10. Notably, 5-HT and th tryptamine derivative 5-methoxytryptamine p ssessed high affinity. Furthermore, as previously reported for the 5-HT_4 receptor, benzamide derivatives including cisapride, BRL 24924 and zacopride bound with fairly high affinity to receptors expressed from the S10 gene. ICS 205930, a tropanyl-indole derivative, which has been reported to be an antagonist at both 5-HT_3 and 5-HT_4 receptors (Bockaert et al., 1992), also bound to these receptor sites. Compounds such as 8-hydroxy-2-(di-n-propylamino)tetralin, ketanserin, sumatriptan and 5-carboxyamidotryptamine were of low affinity having K_i values estimated to be greater than 1 μM. This data would rule out S10 from belonging to other serotonergic receptor subfamilies such as 5-HT_1 and 5-HT_2. Taken together, the complete pharmacological profile also differentiates S10 from the related subtype 5-HT_{4B} (U.S. Serial No., 971,960, filed, November 3, 1992, copending). Although some of the drugs tested also have good affinity for 5-HT_3 receptors, S10 is clearly a 5-HT_4 receptor based upon the binding data and the functional data demonstrating a positive-coupling to adenylate cyclase. Finally, a correlation plot for the binding affinities of 5-HT, cisapride, BRL 24924, zacopride, and ICS 205930 against their functional responses in adenylate cyclase assays from cultured mouse collicular neurons (Dumuis et al., 1989) yielded a correlation coefficient of 0.96 (Figure 8). Thus, the rank order of potency for these key compounds also provides conclusive evidence that S10 encodes a 5-HT_4 receptor.

To examine the ability of S10 clone to couple to adenylate cyclase, Cos-7 cells transiently expressing S10 were tested for the ability to exhibit an increase in basal cAMP release or a decrease in FSK-stimulat d cAMP response. 5-HT (1 μM) had no effect on either

basal or FSK-stimulated adenylate cyclase activity in untransfected or mock-transfected Cos-7 cells (data not shown), indicating that endogen us cyclas -coupled serotonin receptors are not present in untransfected cells. Preliminary studies were carried out by adding only one dose of various drugs in triplicate. Addition of 5-HT (1 μM) to this system caused stimulation of basal cAMP release (CG-7= 0.020 ± 0.002; CG-8= 0.023 ± 0.004 pmol/ml/10min) by about 30 fold for each clone; no inhibition of either the basal or FSK-stimulated cAMP release was observed. On the contrary, addition of 10 μM FSK together with 1 μM 5-HT stimulated cAMP accumulation about 10-fold more than either agent alone (data not shown). For various compounds, full dose-response curves were determined for both clones and the data are summarized in table 4. 5-HT caused a concentration-dependent stimulation of basal adenylate cyclase activity with mean EC_{50}s of 26 ± 3 and 51 ± 7 nM and E_{max}s of 2,107 and 2,598 % basal cAMP release for CG-7 and CG-8 respectively (Figures 9 and 10). Among the tryptamine derivatives tested, 5-MeOT was approximately equipotent with 5-HT in both clones, whereas α-Me-5-HT and 5-CT were about 10 and 200 times respectively less potent than 5-HT at CG-7. On the other hand, the latter two compounds displayed approximately 20 and 30 fold lower affinity than 5-HT respectively for CG-8. The 2-methoxy-4-amino-5-chloro-substituted benzamides (cisapride, BRL-249245 and zacopride) were less potent agonists than 5-HT in stimulating basal cAMP release and displayed different rank order of potency for CG-7 and CG-8. As indicated in table 4 using CG-7, cisapride, BRL-24924 and zacopride exhibited approximately 10, 30 and 100 fold lower potency than 5-HT respectively, whereas at CG-8 these compounds had almost equal affinity. Thus, although not different in binding pr p rties, these subtle differences in affinity in functional assays f

the two "variants" (CG-7 and CG-8) indicate the potential to develop separate therapeutic entities for each separat target. All the agonists tested acted as full agonists with the exception of cisapride, BRL-24924 and zacopride, which also displayed antagonist activity and were therefore partial agonists at both clones, with intrinsic activities ranging between 0.85 and 1.4 (Table 4). ICS-205-930 (100 μM) had similar effect at the two clones and was found to be a silent antagonist causing parallel dextral shifts in the concentration effect curve of 5-HT without altering the maximum response significantly. The estimated K_B value for ICS-205-930 was not significantly different between the two clones (CG-7= 962 ± 244 nM; CG-8= 607 ± 30 nM). Responses to 5-HT were not affected by spiperone or methiothepin (10 μM) in either clone.

Saturation analysis of rat 5-HT_{4A} S10-87 (CG-7) and S10-95 (CG-8) clones and human 5-HT_{4A} clone CG-17 were performed using 10-12 concentrations of [^{3}H]GR113808 (0.005-2.5 nM) and revieled a single saturable site of high affinity for both clones (CG-7: K_d = 0.74 nM, B_{max} = 5.7 pmol/mg membrane protein; CG-8: K_d = 1.0 nM, B_{max} = 3.7 pmol/mg membrane protein; CG-17: K_d = 0.20 nM, B_{max} = 1.8 pmol/mg membrane protein). These preliminary data indicate that although the rat clones (CG-7 and CG-8) have similar affinity for the antagonist [^{3}H]GR113808, the human clone (CG-17) displays approximately 5-fold higher affinity than the rat clones for this ligand. For all three clones nonspecific binding increased linearly with increasing ligand concentration. The degree of specific binding at concentrations of [$_3$H]GR113808 (0.4-0.5 nM) ranged from 80-90%).

The pharmacological binding profile f S10-87 and S10-95 (CG-7, CG-8, respectively) was investigated in

displacement studies using [^{3}H]GR113808 and/or [^{3}H]5-HT. In order to compare CG-17 pharmacology with that previously obtained for the rat clones, CG-7 and CG-8, displacement studies on the human CG-17 clone were performed using [^{3}H]5-HT as the radioligand.

A range of 5-HT$_4$ receptor agonists and antagonists completely inhibited the specific binding of [^{3}H]GR113808 on both the rat CG-7 and CG-8 clones. Affinity values and Hill slopes derived from the curves using computer analysis are presented in Table 5. As previously observed using [^{3}H]5-HT as the radioligand, the rat CG-7 and CG-8 receptors had very similar pharmacology. Of the agonists tested, only those active in 5-HT$_4$ containing preparations (5-HT and 5-MeOT) potently inhibited [^{3}H]GR113808. By contrast, agonists for other 5-HT receptors, for example 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, the 5-HT$_{1D}$ receptor agonist, sumatriptan, the 5-HT$_2$ receptor antagonist, ketanserin, had no effect on [^{3}H]GR113808 binding at concentrations up to 1 μM. The substituted benzamides, cisapride, BRL-24924 and zacopride, partial agonists at 5-HT$_4$ receptor all potently inhibited [^{3}H]GR113808 binding. Specific [^{3}H]GR113808 binding was also inhibited by the 5-HT$_4$ receptor antagonist ICS-205930.

For both the rat CG-7 and CG-8 clones, Hill slopes for the inhibition of [^{3}H]GR113808 binding by 5-HT$_4$ receptor agonists but not the antagonist, ICS-205930, were shallow in the absence of Gpp(NH) with the exception of 5-CT, and α-Me-5-HT. For agonists that had shallow displacement curves, the binding was resolved into high and low affinity components and these are summarized in Table 5. The K_i values obtained for the high affinity state of the receptor using [^{3}H]GR113808 as the radioligand were compatible with the K_i values obtained previously using [^{3}H]5-HT as the radioligand which

labels the high affinity state of the receptor (Table 5 and 6). S me differenc s were observed for the K_i values of high affinity state of CG-7 compared to CG-8 (Table 3) and their nH values. For example, although there were no differences in the K_i values of CG-7 and CG-8, the displacement curve obtained for 5-MeOT using CG-8 clone could not be resolved into two sites. Also the K_i value obtained for the high affinity state of CG-8 using cisapride was approximately 3-fold lower than that obtained for CG-7 previously using [^{3}H]5-HT to directly label the high affinity state of the receptor. We are currently investigating these differences using [^{3}H]5-HT to directly label the high affinity state.

In the presence of 100 μM Gpp(NH)p, competition binding curves for the agonists displaying shallow curves in the absence of Gpp(NH)p were shifted to the right and this is exemplified for 5-HT in Fig. The Hill slopes were increased.

Preliminary results obtained with the human clone (CG-17) using [^{3}H]5-HT as the radioligand in displacement studies are summarized in Table 3. Similar to the rat CG-7 and CG-8 clones, 8-OH-DPAT, sumatriptan and ketanserin were inactive at the CG-17 clone for up to concentration of 1 μM. The differences observed between the human and the rat CG-8 clones were as follows. The biggest difference was observed with α-Me-5-HT which had approximately 100 fold higher affinity for the human CG-17 clone. Zacopride, 5-MeOT and cisapride had about 7-fold, 5-fold and 4-fold higher affinity, respectively for the human clone CG-17.

DISCUSSION

We have identified two cDNA clones encoding the pharmacologically-defined 5-HT_4 receptor. This receptor is expressed at low levels in rat brain if we consider its frequency in the cDNA library ($\leq 1:10^6$). Surprisingly, two receptors differing in their carboxy-terminus regions have been isolated. Since the 3' untranslated nucleotide sequences are also different, these two receptors could be encoded by two different genes or could arise by alternative splicing of pre-mRNA. These two receptors (S10-87 and S10-95) are differentially expressed in rat brain and the physiological relevance of the S10-87 receptor being expressed only in striatum remains to be determined.

The pharmacology binding profile and the functional coupling obtained from cells expressing S10 clones indicate that these genes both encode a pharmacologically-defined 5-HT_4 receptor. The cloned rat CG-7 and CG-8 genes transiently expressed in Cos-7 cells coupled to stimulation of adenylate cyclase. The magnitude of this response (~20-25 fold) was large. With the exception of 5-MeOT, agonist potencies determined from functional assays were less than expected from K_i values obtained from binding assays using [^{3}H]5-HT. Could this result be due to the possibility that the dose of [^{3}H]5-HT used in binding assays measures only the high affinity site of agonists? This is not likely, as it would not account for the data obtained with ICS-205-930 which is a silent antagonist in the present system displaying approximately 6 (CG-8) and 10 (CG-7) fold lower affinity in the functional assay as compared to the binding experiments. It is more likely that differences in experimental conditions used in binding assays compared with those used in the functional

assays (such as membrane vs. cells, buffers and extent of equilibrium achieved) accounts for thes differences.

5-HT responses were resistant to blockade by methiothepin and spiperone (10 μM). As the concentration of these agents exceed their equilibrium dissociation constants for their respective receptor sites by 10-100 fold, it seems that 5-HT_1-like (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}), 5-HT_2 and 5-HT_{4B} receptors can be ruled out. In addition, the weak agonistic activity of 5-CT relative to 5-HT further supports the notion that 5-HT_1-like receptors are probably not involved (Bradley, 1986). The results obtained with the indole agonists reflect those reported at the 5-HT_4 receptor in both the CNS and the periphery (Dumuis et al., 1988; Craig and Clarke, 1990; Eglen et al., 1990; Baxter, Craig and Clarke, 1991). The substituted benzamides, cisapride, BRL-24924 and zacopride acted as partial agonists. Although the benzamides also possess affinity for 5-HT_3 receptors, they lack intrinsic efficacy (Schuurkes et al., 1985; Sanger and King, 1988). Furthermore, the affinity of ICS-205-930 for antagonism of 5-HT response at S10 is 1-3 orders of magnitude lower than that at 5-HT_3 receptors (Richardson et al., 1985) and therefore indicates a binding site different from 5-HT_3 receptor.

As was the case with the rat 5-HT_4 receptor, there are two forms of the human homolog, most likely splice variants differing in the carboxyterminal end of the receptor. Non conservative amino acid substitutions, especially in transmembrane domains 1, 2 and 4 could provide the basis for the pharmacological differences observed between the rat and the human 5-HT_4 receptors (see below). A difference in the functional response is observed between the two rat cl nes: although the

CG-7 construct (S10-87) gives higher levels of receptor expression in COS-7 cells (Bmax of 5.7pmol/mg of protein versus 3.7 pmol/mg for S10-95), in th functional assay, the CG-8 construct (S10-95) reproducibly shows a higher level of cAMP stimulation (2598 ± 154% of basal cAMP release versus 2107 ± 52% for CG-7). This finding could be attributed to the different amino acid sequence in the carboxy terminal tail of the rat receptors, specially since the rat S10-95 isoform carries an additional potential phosphorylation site at position 400, absent in S10-87 (CG-7).

Since the human S10-95 homolog lacks the last 16 carboxy terminal amino acids which carry the phosphorylation site mentioned above in the rat homolog, it will be interesting to check for differences in the level of cAMP stimulation upon activation of the rat and human S10-95 homologs. In the same way, after we get the full length human S10-87, both human isoforms will be compared in binding and functional assays.

All the unique pharmacological characteristics described above define the S10 genes as adenylate cyclase stimulatory "5-HT_4" receptors reported in the literature that are expressed in the ileum (Craig and Clarke, 1990), hippocampus (Shenker et al., 1987), esophagus (Baxter et al., 1991), embryo colliculi neurons (Dumius et al., 1988), atrium (Kaumann et al., 1990), adrenal (Lefebvre et al., 1992) and bladder (Corsi et al., 1991), and distinguish these clones from all other cloned subtypes of 5-HT receptor. Although the binding profile of CG-7 and CG-8 were identical (Table 3), some differences in agonist potency (benzamides in particular) were observed between them in the functional assays. This is not surprising sinc

the amino acid sequences of these two clones are identical, apart fr m the cyt plasmic carboxy tail, a region that is important for G protein-coupling, where the CG-8 receptor carries an extra phosphorylation site. Cisapride, BRL-24924 and zacopride had similar affinities at CG-8 whereas BRL-24924 and zacopride displayed approximately 4 and 15 fold lower affinity than cisapride at CG-7 clone for stimulation of adenylate cyclase. It is noteworthy that tissue differences in the potency of benzamides have been reported (Baxter et al., 1991) and whether this reflects a heterogeneity of 5-HT_4 receptors remains to be investigated.

The data obtained with the rat CG-7 and CG-8 clones and the human CG-17 clone using [^{3}H]GR113808 are very similsr to those reported by Grossmann et al. (1993) and Waeber et al. (1993) with this ligand using guinea pig and human brain tissues. Specific [^{3}H]GR113808 binding readily saturated for all three clones (CG-7, CG-8 and CG-17). Scatchard analysis of specific binding in all three clones revealed the involvement of a single site. Curve fitting analysis showed an equilibrium dissociation of approximately 1 nM for both rat clones (CG-7 and CG-8) whereas this value was about 5-fold lower for the human CG-17 clone (K_d= 0.2 nM). The K_d value obtained for the human CG-17 clone is in excellent agreement with that reported by Waeber et al. (1993) using human brain (0.23-0.37 nM) and is very similar to that of the guinea pig brain tissue (0.13-0.2 nM; Waeber et al., 1993; Grossmann et al., 1993). The K_d value of [^{3}H]GR113808 for rat brain tissue has not been reported, however, it is interesting that the affinity obtained for GR113808 from functional receptor assays in the rat oesophagus (Grossmann et al., 1993) is ab ut 0.3 nM which indicates that this antagonist has similar affinities for the human and the rat tissue

used. The discrepancies between our data and those reported may be due to methodology, r different subtype (brain vs. oesophagus), however, this remains to be investigated.

The rank order of potency of compounds competing for specific [^{3}H]GR113808 are very similar for both CG-7 and CG-8 and is cisapride> 5-HT> BRL-24924> 5-MeOT= ICS205930> zacopride> α-Me-5-HT> 5-CT. This order of potency is different from that observed with guinea pig caudate (Grossmann et al., 1993; cisapride> 5-HT> ICS205930> BRL-24924> zacopride> 5-MeOT> α-Me-5-HT> 5-CT) and human caudate (Waeber et al., 1993; cisapride> ICS205930> BRL-24924> 5-HT> 5-MeOT. Whether these differences are due to species or different population of high affinity state of the receptor in the various preparation, remains to be investigated. Interestingly, the displacement curves for most of the agonists competing for specific [^{3}H]GR113808 were shallow and could be resolved to high and low affinity states. Gpp(NH)p shifted these curves to the right and in the case of 5-HT the Hill coefficient was increased to unity; however for some agonists the shift was not complete. Grossmann et al.(1993) using guinea pig caudate also observed shallow competition curves for some agonist that could be partially shifted by the addition of GTP (Grossmann et al., 1993). However, Waeber did not observe shallow binding curves with the human caudate tissue. These observations indicate that the G protein content of these prepartations may be different which may reflect differences in the coupling of the receptor with a G protein.

Using [^{3}H]5-HT as the radioligand, the affinity values for the human CG-17 clone are in general comparable with that obtained by us pr viously for the rat CG-7 and CG-8 clones with few exceptions. The most striking

differences appears to be for α-Me-5-HT which displays approximat ly 100 fold higher affinity for the human CG-17. However, it has to pointed out that the data are compared with 2 different radioligands and this difference has to be further investigated using the same radioligand for all both the rat and human clones in parallel. 5-MeOT was approximately 3 fold less potent whereas zacopride was about 7 fold more potent at the human CG-17 as compared to the rat clones.

The cloning and expression of genes encoding 5-HT_4 receptors allows, for the first time, the ability to develop subtype selective drugs using radioligand binding assays. It will further provide definitive answers to whether there are significant species differences in the pharmacology of 5-HT_4 receptors. In addition, the intrinsic activity of drugs can be determined from measures of adenylate cyclase activation in these transfected cells. So far, native tissue preparations have shown great disparity in agonist activity. 5-HT_4 receptors have been implicated in a wide variety of functions. Existing drugs such as metaclopramide and cisapride appear to exert a large part of their action through 5-HT_4 receptors (Taniyama et al., 1991; Meulemans and Schurkes, 1992; Flynn et al., 1992). Experience with these agents indicates a clear therapeutic role for 5-HT_4 receptors in the gastrointestinal system for conditions including irritable bowel disease, postoperative ileus, diabetic gastroparesis, emesis, achalasia, hiatal hernia, and esophageal spasm. In addition, 5-HT_4 receptors have been described functionally in the heart (Kaumann, 1992), adrenal (Lefebvre et al., 1992), and bladder (Corsi et al., 1991) indicating possible roles in cardiac rate and force of contraction, endocrine control of cortisol secretion, and urinary incontinence or spasticity. 5-HT_4 recept rs have also been

described in the brain, particularly in areas such as the hippocampus, in which we have localized the gene encoding 5-HT_4 receptors (S10-87), indicating a potential role in cognitive enhancement (Bockaert et al., 1992). As more specific pharmacological tools are developed, additional therapeutic indications will certainly be uncovered.

Table I: % TM homology of the S10 receptor with other 7 TM receptors

SEROTONIN		ADRENERGIC		DOPAMINE		PEPTIDE	
5-HT_{1A}	Hu 44	Alpha-1A	Hu 45	D_1	Hu 43	Subst K	Hu 25
5-$HT_{1D\alpha}$	Hu 40	Alpha-1B	Hu 43	D_2	Hu 42	TSH	2[redacted]
5-$HT_{1D\beta}$	Hu 41	Alpha-1C	Hu 40	D_3	Rt 46		
5-HT_{1E}	Hu 41	Alpha-2A	Hu 42	D_4	Hu 45		
5-HT_{1F}	Hu 41	Alpha-2B	Hu 40	D_6	Hu 45		
5-HT_2	Hu 42	Alpha-2C	Hu 40				
5-HT_{1C}	Hu 44						
		Beta-1	Hu 46	HISTAMINE		MUSCARINIC	
5-HT Dro S	43	Beta-2	Hu 44				
5-HT Dro I_A	40	Beta-3	Hu 42	H_1	Bov 36	m1	35
5-HT Dro I_B	41			H_2	Hu 46		
5-HT_{4B}	Hu 44						
				ADENOSINE			
				A1	Rat 32		
				A2	Hu 31		

TABLE 2

Primers	Cortex	Cerebellum	Brain Stem	Hippocampus	Olfactory Bulb	Striatum	Thalamus
TM3-5	+	-	+	+	+	+	+
S10-87	ND	-	-	-	-	+	-
S10-95	ND	-	+	+	+	+	+

Table 2: PCR localization of the S10 mRNA in 7 different part of the rat brain. The TM3-5 primers do not differentiate between clones S10-87 and S10-95. The S10-87 primers were designed from the nucleotide sequences coding for the TM 6 domain common to both receptors and for the carboxy terminus end specific to S10-87. In the same way, the S10-95 primers are specific for S10-95.

Table 3. Binding affinities of key ligands for the identification of S10 (CG-7 and CG-8) as a 5-HT_4 receptor. Affinity constants (Ki; nM) of drugs competing for S10 label d with 10 nM [3H]5-HT were determined to pharmacologically define the encoded receptor as 5-HT_4. Ki (nM) values were calculated using the Cheng-Prusoff equation or estimated to be >1000 nM based upon one point displacements using a drug concentration of 1 uM. Affinity constants are expressed as the mean ± SEM (n≥2). Ki values estimated to be >1000 wer determined according to one point displacements studies at a concentration of 1 uM. (n=2 except BRL 24924 tested at CG-8: n=1)

CHARACTERISATION OF CLONE S-10

Saturation Analysis: Kd = 7.87 ± 0.06 nM
Bmax = 1,938 fmol/ mg prot

Pharmacological profile:

DRUG	CG-7	CG-8
5-HT	8.6 ± 0.6	6.4 ± 0.5
Cisapride	10.9 ± 0.3	ND
5-MeOT	27.5 ± 5	ND
BRL 24924	27.7 ± 5	21.1*
ICS 205930	115 ± 12	138 ± 26
Zacopride	130 ± 10	136 ± 5
8OHDPAT	>1000	ND
Ketanserin	>1000	ND
Sumatriptan	>1000	ND
5-CT	>1000	ND

* n = 1 ND = not determined

Table 4. Pharmacological profile for the cAMP response using the human 5-HT_{4A} (CG-7 and CG-8) receptor transiently expressed in Cos-7 cells, comparison with the binding data obtained with CG-7 clone using [^{3}H]5-HT. cAMP measurements on intact cells were as described under Methods and Mat rials. EC_{50} values (concentration producing the half-maximal effect) were derived fr m the analysis of full dose-response curves. Maximum response produced by each drug was normalized to the 5-HT maximum response which is indicated as having an intrinsic activity of 1.0. Data are means ± S.E.M. of three separate experiments. The apparent dissociation constant of antagonist (K_B) (ICS 205930) was calculated according to the formula: $K_B = [B]/(A'/A)-1]$, where [B] is the conc ntrati n of antagonist, A' and A the EC_{50} values of agonist measured respectively in the presence and in the absence of antagonist (Furchgott, 1972).

	EC_{50} or K_B (nM)		I. A.*		K_i (nM)	K_i (nM)
DRUG	CG-7	CG-8	CG-7	CG-8	CG-7	CG-8
5-MeOT	21 ± 6	31 ± 13	1.0	1.0	27 ± 5	ND
5-HT	26 ± 3	51 ± 7	1.0	1.0	8.6 ± 0.6	6.4 ± 0.5
Cisapride	191 ± 26	413 ± 199	1.2	1.4	11 ± 0.3	ND
α-Me-5-HT	250 ± 91	1,038 ± 31	0.90	1.0	ND	ND
BRL-24924	736 ± 129	250 ± 25	1.1	0.9	28 ± 5	21
Zacopride	2,740 ± 274	239 ± 33	1.1	1.0	130 ± 10	136 ± 5
5-CT	5,570± 808	1,411 ± 211	0.85	1.2	>1,000	ND
ICS-205930	962 ± 244	607 ± 30	0	0	115 ± 12	138 ± 26

ND, n t determined.
Maximum response to 5-HT in Cos-7 cells transiently transfected with CG-7 and CG-8 genes was:
CG-7= 2,107 ±.52 % of basal cAMP release
CG-8= 2,598 ± 154% of basal cAMP release

Cisapride, BRL-24924 and zacopride had no antagonist activity and ICS-205930 had no intrinsic agonist activity.

Table 5. The affinities of various compounds that compete for 0.2-0.4 nM [^{3}H]GR113808 binding in membranes of COS-7 cells transiently transfected with rat clones CG-7 and CG-8.

COMPOUNDS	CG-7	CG-8
5-HT	237 K_H=2.6, BH=22% K_L=357 nH=0.62	116 KH=2.5, BH=24% KL=197 nH=0.67
5-CT	>10,000	>10,000
5-Me-OT	438 KH=14, BH=17% KL=658 nH=0.57	518 KH=, BH= KL= nH=0.66
BRL-24924	189 KH=37, BH=34% KL=371 nH=0.85	188 KH=23, BH=32% KL=373 nH=0.81
ZACOPRIDE	729 KH=424, BH=68% KL=2,757 nH=0.80	820 KH=, BH= KL= nH=0.85
d-LSD	>10,000	>10,000
CISAPRIDE	ND	84 KH=2.7, BH=11% KL=105 nH=0.83
ICS205930	ND	529
α-Me-5-HT	2,255	1,855

Affinity estimates are given as K_I values (nM) using the computer program "Accucomp" (Lundon S ftware). Values are from a single experiment. K_H and K_L values indicate the affinity in nM for the high affinity and low affinity state of the receptor, B_H is the percentage of high affinity sites and nH is the Hill coefficient.
ND, not determined.

Tabl 6. Binding affinities of key ligands for the identification of the human CG-17 clone as a 5-HT_{4A} receptor.

COMPOUND	K_i (nM)
5-HT	4.2
5-MeOT	71
5-CT	>10,000
Cisapride	12
α-Me-5-HT	1.6
BRL-24924	21
Zacopride	17
Sumatriptan	>1,000
8-OH-DPAT	>1,000
Ketanserin	>1,000

Affinity constants (Ki, nM) of drugs competing for CG-17 cloned labeled with 10 nM [^{3}H]5-HT were calculated using the Cheng-Prusoff equation or estimated to be greater than 1,000 nM based upon one point displacement using a drug concentration of 1 μM. Values are from a single experiment.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SYNAPTIC PHARMACEUTICAL CORPORATION

(ii) TITLE OF INVENTION: DNA ENCODING 5-HT4 SEROTONIN RECEPTORS AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: COOPER & DUNHAM
(B) STREET: 30 ROCKEFELLER PLAZA
(C) CITY: NEW YORK
(D) STATE: NEW YORK
(F) ZIP: 10112

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Patentin Release #1.24

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: White, P., John
(B) REGISTRATION NUMBER: 28,678
(C) REFERENCE/DOCKET NUMBER: 42667-A-PCT/JPW/TEP

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 977-9550
(B) TELEFAX: (212) 664-0525
(C) TELEX: 422523 COOP UI

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1642 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: brain

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: rat brain
(B) CLONE: S10-87

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 101..1261
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TGGGCACTGA  CATCCAACGT  ACTCATGCCC  ATTTCCTGTA  ATG GAC AGA CTT GAT         115
                                                Met Asp Arg Leu Asp
                                                  1               5

GCT AAT GTG AGT TCC AAC GAG GGT TTC GGG TCT GTG GAG AAG GTC GTA           163
Ala Asn Val Ser Ser Asn Glu Gly Phe Gly Ser Val Glu Lys Val Val
                    10                  15                  20

CTG CTC ACG TTC TTC GCA ATG GTT ATC CTG ATG GCC ATC CTG GGC AAC           211
Leu Leu Thr Phe Phe Ala Met Val Ile Leu Met Ala Ile Leu Gly Asn
                25                  30                  35

CTG CTG GTG ATG GTT GCT GTG TGC AGG GAC AGG CAG CTC AGG AAA ATA           259
Leu Leu Val Met Val Ala Val Cys Arg Asp Arg Gln Leu Arg Lys Ile
            40                  45                  50

AAA ACC AAT TAT TTC ATT GTG TCT CTT GCC TTT GCT GAT CTG CTG GTT           307
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Lys	Thr	Asn	Tyr	Phe	Ile	Val	Ser	Leu	Ala	Phe	Ala	Asp	Leu	Leu	Val
	55					60					65				
TCG	GTG	CTG	GTG	AAT	GCC	TTC	GGT	GCC	ATT	GAG	TTG	GTC	CAA	GAC	ATC	355
Ser	Val	Leu	Val	Asn	Ala	Phe	Gly	Ala	Ile	Glu	Leu	Val	Gln	Asp	Ile	
70					75					80					85	
TGG	TTT	TAT	GGG	GAG	ATG	TTT	TGC	CTG	GTC	CGG	ACC	TCT	CTG	GAT	GTC	403
Trp	Phe	Tyr	Gly	Glu	Met	Phe	Cys	Leu	Val	Arg	Thr	Ser	Leu	Asp	Val	
				90					95					100		
CTA	CTC	ACC	ACA	GCA	TCA	ATT	TTT	CAC	CTC	TGC	TGC	ATT	TCC	CTG	GAT	451
Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His	Leu	Cys	Cys	Ile	Ser	Leu	Asp	
			105					110					115			
AGG	TAT	TAT	GCC	ATC	TGC	TGT	CAA	CCT	TTG	GTT	TAT	AGA	AAC	AAG	ATG	499
Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro	Leu	Val	Tyr	Arg	Asn	Lys	Met	
		120					125					130				
ACC	CCT	CTA	CGC	ATC	GCA	TTA	ATG	CTG	GGA	GGC	TGC	TGG	GTC	ATT	CCC	547
Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu	Gly	Gly	Cys	Trp	Val	Ile	Pro	
	135					140					145					
ATG	TTT	ATA	TCT	TTT	CTC	CCC	ATA	ATG	CAA	GGC	TGG	AAC	AAC	ATC	GGC	595
Met	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met	Gln	Gly	Trp	Asn	Asn	Ile	Gly	
150					155					160					165	
ATA	GTT	GAT	GTG	ATA	GAG	AAA	AGG	AAA	TTC	AAC	CAC	AAC	TCT	AAC	TCT	643
Ile	Val	Asp	Val	Ile	Glu	Lys	Arg	Lys	Phe	Asn	His	Asn	Ser	Asn	Ser	
				170					175					180		
ACA	TTC	TGT	GTC	TTC	ATG	GTC	AAC	AAG	CCC	TAT	GCC	ATC	ACC	TGC	TCT	691
Thr	Phe	Cys	Val	Phe	Met	Val	Asn	Lys	Pro	Tyr	Ala	Ile	Thr	Cys	Ser	
			185					190					195			
GTG	GTG	GCC	TTC	TAC	ATC	CCG	TTT	CTC	CTC	ATG	GTG	CTG	GCC	TAT	TAC	739
Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu	Leu	Met	Val	Leu	Ala	Tyr	Tyr	
		200					205					210				
CGT	ATC	TAT	GTC	ACT	GCT	AAG	GAG	CAT	GCC	CAG	CAG	ATC	CAG	ATG	TTA	787
Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His	Ala	Gln	Gln	Ile	Gln	Met	Leu	
	215					220					225					
CAA	CGG	GCA	GGA	GCC	ACC	TCT	GAA	AGC	AGG	CCC	CAG	ACA	GCT	GAC	CAG	835
Gln	Arg	Ala	Gly	Ala	Thr	Ser	Glu	Ser	Arg	Pro	Gln	Thr	Ala	Asp	Gln	

230					235					240					245	
CAC	AGC	ACA	CAT	CGC	ATG	CGG	ACA	GAG	ACC	AAA	GCA	GCC	AAG	ACT	TTA	883
His	Ser	Thr	His	Arg	Met	Arg	Thr	Glu	Thr	Lys	Ala	Ala	Lys	Thr	Leu	
				250					255					260		
TGT	GTC	ATC	ATG	GGC	TGC	TTC	TGT	TTC	TGC	TGG	GCC	CCC	TTC	TTT	GTC	931
Cys	Val	Ile	Met	Gly	Cys	Phe	Cys	Phe	Cys	Trp	Ala	Pro	Phe	Phe	Val	
			265					270					275			
ACC	AAT	ATT	GTG	GAC	CCT	TTC	ATA	GAC	TAC	ACT	GTG	CCC	GAG	AAG	GTG	979
Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp	Tyr	Thr	Val	Pro	Glu	Lys	Val	
		280					285					290				
TGG	ACT	GCT	TTC	CTC	TGG	CTT	GGC	TAT	ATC	AAT	TCA	GGG	TTG	AAC	CCT	1027
Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr	Ile	Asn	Ser	Gly	Leu	Asn	Pro	
	295					300					305					
TTT	CTC	TAT	GCC	TTC	TTG	AAT	AAG	TCT	TTC	AGA	CGT	GCC	TTC	CTT	ATC	1075
Phe	Leu	Tyr	Ala	Phe	Leu	Asn	Lys	Ser	Phe	Arg	Arg	Ala	Phe	Leu	Ile	
310					315					320					325	
ATC	CTC	TGC	TGT	GAT	GAT	GAG	CGC	TAC	AAA	AGA	CCC	CCC	ATT	CTG	GGC	1123
Ile	Leu	Cys	Cys	Asp	Asp	Glu	Arg	Tyr	Lys	Arg	Pro	Pro	Ile	Leu	Gly	
				330					335					340		
CAG	ACT	GTC	CCC	TGT	TCA	ACC	ACA	ACC	ATT	AAT	GGA	TCC	ACT	CAT	GTG	1171
Gln	Thr	Val	Pro	Cys	Ser	Thr	Thr	Thr	Ile	Asn	Gly	Ser	Thr	His	Val	
			345					350					355			
CTA	AGG	TAT	ACA	GTT	TTG	CAT	AGT	GGT	CAA	CAC	CAG	GAA	CTG	GAG	AAG	1219
Leu	Arg	Tyr	Thr	Val	Leu	His	Ser	Gly	Gln	His	Gln	Glu	Leu	Glu	Lys	
		360					365					370				
TTG	CCC	ATA	CAC	AAT	GAC	CCA	GAG	TCC	CTG	GAA	TCA	TGC	TTT			1261
Leu	Pro	Ile	His	Asn	Asp	Pro	Glu	Ser	Leu	Glu	Ser	Cys	Phe			
	375					380					385					

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TTTCAGAACC GTGTGGGGAT TTGAGATGTC ATCAGACCCA GTGTCTTACC CAGAGCCCAA 1501

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 387 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asp	Arg	Leu	Asp	Ala	Asn	Val	Ser	Ser	Asn	Glu	Gly	Phe	Gly	Ser
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Val	Glu	Lys	Val	Val	Leu	Leu	Thr	Phe	Phe	Ala	Met	Val	Ile	Leu	Met
			20					25					30		
Ala	Ile	Leu	Gly	Asn	Leu	Leu	Val	Met	Val	Ala	Val	Cys	Arg	Asp	Arg
		35					40					45			
Gln	Leu	Arg	Lys	Ile	Lys	Thr	Asn	Tyr	Phe	Ile	Val	Ser	Leu	Ala	Phe
	50					55					60				
Ala	Asp	Leu	Leu	Val	Ser	Val	Leu	Val	Asn	Ala	Phe	Gly	Ala	Ile	Glu
65					70					75					80
Leu	Val	Gln	Asp	Ile	Trp	Phe	Tyr	Gly	Glu	Met	Phe	Cys	Leu	Val	Arg
				85					90					95	
Thr	Ser	Leu	Asp	Val	Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His	Leu	Cys
			100					105					110		
Cys	Ile	Ser	Leu	Asp	Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro	Leu	Val
		115					120					125			
Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu	Gly	Gly
	130					135					140				

Cys Trp Val Ile Pro Met Phe Ile Ser Phe Leu Pro Ile Met Gln Gly
145 150 155 160

Trp Asn Asn Ile Gly Ile Val Asp Val Ile Glu Lys Arg Lys Phe Asn
165 170 175

His Asn Ser Asn Ser Thr Phe Cys Val Phe Met Val Asn Lys Pro Tyr
180 185 190

Ala Ile Thr Cys Ser Val Val Ala Phe Tyr Ile Pro Phe Leu Leu Met
195 200 205

Val Leu Ala Tyr Tyr Arg Ile Tyr Val Thr Ala Lys Glu His Ala Gln
210 215 220

Gln Ile Gln Met Leu Gln Arg Ala Gly Ala Thr Ser Glu Ser Arg Pro
225 230 235 240

Gln Thr Ala Asp Gln His Ser Thr His Arg Met Arg Thr Glu Thr Lys
245 250 255

Ala Ala Lys Thr Leu Cys Val Ile Met Gly Cys Phe Cys Phe Cys Trp
260 265 270

Ala Pro Phe Phe Val Thr Asn Ile Val Asp Pro Phe Ile Asp Tyr Thr
275 280 285

Val Pro Glu Lys Val Trp Thr Ala Phe Leu Trp Leu Gly Tyr Ile Asn
290 295 300

Ser Gly Leu Asn Pro Phe Leu Tyr Ala Phe Leu Asn Lys Ser Phe Arg
305 310 315 320

Arg Ala Phe Leu Ile Ile Leu Cys Cys Asp Asp Glu Arg Tyr Lys Arg
325 330 335

Pro Pro Ile Leu Gly Gln Thr Val Pro Cys Ser Thr Thr Thr Ile Asn
340 345 350

Gly Ser Thr His Val Leu Arg Tyr Thr Val Leu His Ser Gly Gln His
355 360 365

Gln Glu Leu Glu Lys Leu Pro Ile His Asn Asp Pro Glu Ser Leu Glu
370 375 380

Ser Cys Phe

385

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1622 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: brain

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: rat brain
(B) CLONE: S10-95

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 50..1267
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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                                                           Met Asp
                                                            1

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Arg Leu Asp Ala Asn Val Ser Ser Asn Glu Gly Phe Gly Ser Val Glu
        5                  10                  15

AAG GTC GTA CTG CTC ACC TTC TTC GCA ATG GTT ATC CTG ATG GCC ATC          151
Lys Val Val Leu Leu Thr Phe Phe Ala Met Val Ile Leu Met Ala Ile
    20                  25                  30

CTG GGC AAC CTG CTG GTG ATG GTT GCT GTG TGC AGG GAC AGG CAG CTC          199
Leu Gly Asn Leu Leu Val Met Val Ala Val Cys Arg Asp Arg Gln Leu
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35 40 45 50

AGG	AAA	ATA	AAA	ACC	AAT	TAT	TTC	ATT	GTG	TCT	CTT	GCC	TTT	GCT	GAT	247
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				55					60					65		
CTG	CTG	GTT	TCG	GTG	CTG	GTG	AAT	GCC	TTC	GGT	GCC	ATT	GAG	TTG	GTC	295
Leu	Leu	Val	Ser	Val	Leu	Val	Asn	Ala	Phe	Gly	Ala	Ile	Glu	Leu	Val	
			70					75					80			
CAA	GAC	ATC	TGG	TTT	TAT	GGG	GAG	ATG	TTT	TGC	CTG	GTC	CGG	ACC	TCT	343
Gln	Asp	Ile	Trp	Phe	Tyr	Gly	Glu	Met	Phe	Cys	Leu	Val	Arg	Thr	Ser	
		85					90					95				
CTG	GAT	GTC	CTA	CTC	ACC	ACA	GCA	TCA	ATT	TTT	CAC	CTC	TGC	TGC	CTT	391
Leu	Asp	Val	Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His	Leu	Cys	Cys	Leu	
	100					105					110					
TCC	CTG	GAT	AGG	TAT	TAT	GCC	ATC	TGC	TGT	CAA	CCT	TTG	GTT	TAT	AGA	439
Ser	Leu	Asp	Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro	Leu	Val	Tyr	Arg	
115					120					125					130	
AAC	AAG	ATG	ACC	CCT	CTA	CGC	ATC	GCA	TTA	ATG	CTG	GGA	GGC	TGC	TGG	487
Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu	Gly	Gly	Cys	Trp	
				135					140					145		
GTC	ATT	CCC	ATG	TTT	ATA	TCT	TTT	CTC	CCC	ATA	ATG	CAA	GGC	TGG	AAC	535
Val	Ile	Pro	Met	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met	Gln	Gly	Trp	Asn	
			150					155					160			
AAC	ATC	GGC	ATA	GTT	GAT	GTG	ATA	GAG	AAA	AGG	AAA	TTC	AAC	CAC	AAC	583
Asn	Ile	Gly	Ile	Val	Asp	Val	Ile	Glu	Lys	Arg	Lys	Phe	Asn	His	Asn	
		165					170					175				
TCT	AAC	TCT	ACA	TTC	TGT	GTC	TTC	ATG	GTC	AAC	AAG	CCC	TAT	GCC	ATC	631
Ser	Asn	Ser	Thr	Phe	Cys	Val	Phe	Met	Val	Asn	Lys	Pro	Tyr	Ala	Ile	
	180					185					190					
ACC	TGC	TCT	GTG	GTG	GCC	TTC	TAC	ATC	CCG	TTT	CTC	CTC	ATG	GTG	CTG	679
Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu	Leu	Met	Val	Leu	
195					200					205					210	
GCC	TAT	TAC	CGT	ATC	TAT	GTC	ACT	GCT	AAG	GAG	CAT	GCC	CAG	CAG	ATC	727
Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His	Ala	Gln	Gln	Ile	
				215					220					225		

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CAG ATG TTA CAA CGG GCA GGA GCC ACC TCT GAA AGC AGG CCC CAG ACA        775
Gln Met Leu Gln Arg Ala Gly Ala Thr Ser Glu Ser Arg Pro Gln Thr
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GCT GAC CAG CAC AGC ACA CAT CGC ATG CGG ACA GAG ACC AAA GCA GCC        823
Ala Asp Gln His Ser Thr His Arg Met Arg Thr Glu Thr Lys Ala Ala
            245                 250                 255

AAG ACT TTA TGT GTC ATC ATG GGC TGC TTC TGT TTC TGC TGG GCC CCC        871
Lys Thr Leu Cys Val Ile Met Gly Cys Phe Cys Phe Cys Trp Ala Pro
        260                 265                 270

TTC TTT GTC ACC AAT ATT GTG GAC CCT TTC ATA GAC TAC ACT GTG CCC        919
Phe Phe Val Thr Asn Ile Val Asp Pro Phe Ile Asp Tyr Thr Val Pro
275                 280                 285                 290

GAG AAG GTG TGG ACT GCT TTC CTC TGG CTT GGC TAT ATC AAT TCA GGG        967
Glu Lys Val Trp Thr Ala Phe Leu Trp Leu Gly Tyr Ile Asn Ser Gly
                295                 300                 305

TTG AAC CCT TTT CTC TAT GCC TTC TTG AAT AAG TCT TTC AGA CGT GCC       1015
Leu Asn Pro Phe Leu Tyr Ala Phe Leu Asn Lys Ser Phe Arg Arg Ala
            310                 315                 320

TTC CTT ATC ATC CTC TGC TGT GAT GAT GAG CGC TAC AAA AGA CCC CCC       1063
Phe Leu Ile Ile Leu Cys Cys Asp Asp Glu Arg Tyr Lys Arg Pro Pro
        325                 330                 335

ATT CTG GGC CAG ACT GTC CCC TGT TCA ACC ACA ACC ATT AAT GGA TCC       1111
Ile Leu Gly Gln Thr Val Pro Cys Ser Thr Thr Thr Ile Asn Gly Ser
    340                 345                 350

ACT CAT GTG CTA AGG GAT ACA GTG GAA TGT GGT GGC CAA TGG GAG AGT       1159
Thr His Val Leu Arg Asp Thr Val Glu Cys Gly Gly Gln Trp Glu Ser
355                 360                 365                 370

CGG TGT CAC CTC ACA GCA ACT TCT CCT TTG GTG GCT GCT CAG CCA GTG       1207
Arg Cys His Leu Thr Ala Thr Ser Pro Leu Val Ala Ala Gln Pro Val
                375                 380                 385

ATA CGT AGG CCC CAG GAC AAT GAC CTA GAA GAC AGC TGT AGC TTG AAA       1255
Ile Arg Arg Pro Gln Asp Asn Asp Leu Glu Asp Ser Cys Ser Leu Lys
            390                 395                 400

AGA AGC CAG TCC TAAGCTGCTA     CTTCGGTGTA     TGTGGCTGCC     CCTGGCACTT      1307
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Arg Ser Gln Ser
405

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CCAGTCCCCT TTTGCTGTCT CCAGTCTGTC TTGTAAATGT TTACCGTGTT CGATTTTCAG 1547

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TTGATCACAC TTCTT 1622

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 406 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Arg Leu Asp Ala Asn Val Ser Ser Asn Glu Gly Phe Gly Ser
1 5 10 15

Val Glu Lys Val Val Leu Leu Thr Phe Phe Ala Met Val Ile Leu Met
20 25 30

Ala Ile Leu Gly Asn Leu Leu Val Met Val Ala Val Cys Arg Asp Arg
35 40 45

Gln Leu Arg Lys Ile Lys Thr Asn Tyr Phe Ile Val Ser Leu Ala Phe
50 55 60

Ala Asp Leu Leu Val Ser Val Leu Val Asn Ala Phe Gly Ala Ile Glu
65 70 75 80

Leu Val Gln Asp Ile Trp Phe Tyr Gly Glu Met Phe Cys Leu Val Arg
85 90 95

Thr	Ser	Leu	Asp	Val	Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His	Leu	Cys
			100					105					110		
Cys	Leu	Ser	Leu	Asp	Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro	Leu	Val
		115					120					125			
Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu	Gly	Gly
	130					135					140				
Cys	Trp	Val	Ile	Pro	Met	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met	Gln	Gly
145					150					155					160
Trp	Asn	Asn	Ile	Gly	Ile	Val	Asp	Val	Ile	Glu	Lys	Arg	Lys	Phe	Asn
				165					170					175	
His	Asn	Ser	Asn	Ser	Thr	Phe	Cys	Val	Phe	Met	Val	Asn	Lys	Pro	Tyr
			180					185					190		
Ala	Ile	Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu	Leu	Met
		195					200					205			
Val	Leu	Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His	Ala	Gln
	210					215					220				
Gln	Ile	Gln	Met	Leu	Gln	Arg	Ala	Gly	Ala	Thr	Ser	Glu	Ser	Arg	Pro
225					230					235					240
Gln	Thr	Ala	Asp	Gln	His	Ser	Thr	His	Arg	Met	Arg	Thr	Glu	Thr	Lys
				245					250					255	
Ala	Ala	Lys	Thr	Leu	Cys	Val	Ile	Met	Gly	Cys	Phe	Cys	Phe	Cys	Trp
			260					265					270		
Ala	Pro	Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp	Tyr	Thr
		275					280					285			
Val	Pro	Glu	Lys	Val	Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr	Ile	Asn
	290					295					300				
Ser	Gly	Leu	Asn	Pro	Phe	Leu	Tyr	Ala	Phe	Leu	Asn	Lys	Ser	Phe	Arg
305					310					315					320
Arg	Ala	Ph	Leu	Ile	Ile	Leu	Cys	Cys	Asp	Asp	Glu	Arg	Tyr	Lys	Arg
				325					330					335	

Pro Pro Ile Leu Gly Gln Thr Val Pro Cys Ser Thr Thr Thr Ile Asn
340 345 350

Gly Ser Thr His Val Leu Arg Asp Thr Val Glu Cys Gly Gly Gln Trp
355 360 365

Glu Ser Arg Cys His Leu Thr Ala Thr Ser Pro Leu Val Ala Ala Gln
370 375 380

Pro Val Ile Arg Arg Pro Gln Asp Asn Asp Leu Glu Asp Ser Cys Ser
385 390 395 400

Leu Lys Arg Ser Gln Ser
405

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 536 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: brain

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: human brain
(B) CLONE: S10 PCR

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..534
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTG GTC TAT AGG AAC AAG ATG ACC CCT CTG CGC ATC GCA TTA ATG CTG 48

```
Leu  Val  Tyr  Arg  Asn  Lys  Met  Thr  Pro  Leu  Arg  Ile  Ala  Leu  Met  Leu
 1                        5                      10                     15

GGA  GGC  TGC  TGG  GTC  ATC  CCC  ACG  TTT  ATT  TCT  TTT  CTC  CCT  ATA  ATG      96
Gly  Gly  Cys  Trp  Val  Ile  Pro  Thr  Phe  Ile  Ser  Phe  Leu  Pro  Ile  Met
               20                      25                     30

CAA  GGC  TGG  AAT  AAC  ATT  GGC  ATA  ATT  GAT  TTG  ATA  GAA  AAG  AGG  AAG      144
Gln  Gly  Trp  Asn  Asn  Ile  Gly  Ile  Ile  Asp  Leu  Ile  Glu  Lys  Arg  Lys
          35                      40                     45

TTC  AAC  CAG  AAC  TCT  AAC  TCT  ACG  TAC  TGT  GTC  TTC  ATG  GTC  AAC  AAG      192
Phe  Asn  Gln  Asn  Ser  Asn  Ser  Thr  Tyr  Cys  Val  Phe  Met  Val  Asn  Lys
     50                      55                     60

CCC  TAC  GCC  ATC  ACC  TGC  TCT  GTG  GTG  GCC  TTC  TAC  ATC  CCA  TTT  CTC      240
Pro  Tyr  Ala  Ile  Thr  Cys  Ser  Val  Val  Ala  Phe  Tyr  Ile  Pro  Phe  Leu
65                      70                     75                     80

CTC  ATG  GTG  CTG  GCC  TAT  TAC  CGC  ATC  TAT  GTC  ACA  GCT  AAG  GAG  CAT      288
Leu  Met  Val  Leu  Ala  Tyr  Tyr  Arg  Ile  Tyr  Val  Thr  Ala  Lys  Glu  His
               85                      90                     95

GCC  CAT  CAG  ATC  CAG  ATG  TTA  CAA  CGG  GCA  GGA  GCC  TCC  TCC  GAG  AGC      336
Ala  His  Gln  Ile  Gln  Met  Leu  Gln  Arg  Ala  Gly  Ala  Ser  Ser  Glu  Ser
          100                     105                    110

AGG  CCT  CAG  TCG  GCA  GAC  CAG  CAT  AGC  ACT  CAT  CCG  ATG  AGG  ACA  GAG      384
Arg  Pro  Gln  Ser  Ala  Asp  Gln  His  Ser  Thr  His  Pro  Met  Arg  Thr  Glu
     115                     120                    125

ACC  AAA  GCA  GCC  AAG  ACC  CTG  TGC  ATC  ATC  ATG  GGT  TGC  TTC  TGC  CTC      432
Thr  Lys  Ala  Ala  Lys  Thr  Leu  Cys  Ile  Ile  Met  Gly  Cys  Phe  Cys  Leu
     130                     135                    140

TGC  TGG  GCA  CCA  TTC  TTT  GTC  ACC  AAT  ATT  GTG  GAT  CCT  TTC  ATA  GAC      480
Cys  Trp  Ala  Pro  Phe  Phe  Val  Thr  Asn  Ile  Val  Asp  Pro  Phe  Ile  Asp
145                     150                    155                    160

TAC  ACT  GTC  CCT  GGG  CAG  GTG  TGG  ACT  GCT  TTC  CTC  TGG  CTC  GGC  TAT      528
Tyr  Thr  Val  Pro  Gly  Gln  Val  Trp  Thr  Ala  Phe  Leu  Trp  Leu  Gly  Tyr
               165                     170                    175

ATC  AAT  TC                                                                   536
Ile  Asn
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 178 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu	Val	Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu
1				5					10					15	
Gly	Gly	Cys	Trp	Val	Ile	Pro	Thr	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met
			20					25					30		
Gln	Gly	Trp	Asn	Asn	Ile	Gly	Ile	Ile	Asp	Leu	Ile	Glu	Lys	Arg	Lys
		35					40					45			
Phe	Asn	Gln	Asn	Ser	Asn	Ser	Thr	Tyr	Cys	Val	Phe	Met	Val	Asn	Lys
	50					55					60				
Pro	Tyr	Ala	Ile	Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu
65					70					75					80
Leu	Met	Val	Leu	Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His
				85					90					95	
Ala	His	Gln	Ile	Gln	Met	Leu	Gln	Arg	Ala	Gly	Ala	Ser	Ser	Glu	Ser
			100					105					110		
Arg	Pro	Gln	Ser	Ala	Asp	Gln	His	Ser	Thr	His	Pro	Met	Arg	Thr	Glu
		115					120					125			
Thr	Lys	Ala	Ala	Lys	Thr	Leu	Cys	Ile	Ile	Met	Gly	Cys	Phe	Cys	Leu
	130					135					140				
Cys	Trp	Ala	Pro	Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp
145					150					155					160
Tyr	Thr	Val	Pro	Gly	Gln	Val	Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr
				165					170					175	

Ile Asn

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1316 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: Brain

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Human Brain
(B) CLONE: S10-87

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 7..1170

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CCTGTA  ATG GAC AAA CTT GAT GCT AAT GTG AGT TCT GAG GAG GGT TTC          48
        Met Asp Lys Leu Asp Ala Asn Val Ser Ser Glu Glu Gly Phe
          1               5                  10

GGG TCA GTG GAG AAG GTG GTG CTG CTC ACG TTT CTC TCG ACG GTT ATC          96
Gly Ser Val Glu Lys Val Val Leu Leu Thr Phe Leu Ser Thr Val Ile
 15                  20                  25                  30

CTG ATG GCC ATC TTG GGG AAC CTG CTG GTG ATG GTG GCT GTG TGC TGG         144
Leu Met Ala Ile Leu Gly Asn Leu Leu Val Met Val Ala Val Cys Trp
                35                  40                  45

GAC AGG CAG CTC AGG AAA ATA AAA ACA AAT TAT TTC ATT GTA TCT CTT         192
Asp Arg Gln Leu Arg Lys Ile Lys Thr Asn Tyr Phe Ile Val Ser Leu
            50                  55                  60
```

GCT	TTT	GCG	GAT	CTG	CTG	GTT	TCG	GTG	CTG	GTG	ATG	CCC	TTT	GGT	GCC	240
Ala	Phe	Ala	Asp	Leu	Leu	Val	Ser	Val	Leu	Val	Met	Pro	Phe	Gly	Ala	
		65					70					75				
ATT	GAG	CTG	GTT	CAA	GAC	ATC	TGG	ATT	TAT	GGG	GAG	GTG	TTT	TGT	CTT	288
Ile	Glu	Leu	Val	Gln	Asp	Ile	Trp	Ile	Tyr	Gly	Glu	Val	Phe	Cys	Leu	
	80					85					90					
GTT	CGG	ACA	TCT	CTG	GAC	GTC	CTG	CTC	ACA	ACG	GCA	TCG	ATT	TTT	CAC	336
Val	Arg	Thr	Ser	Leu	Asp	Val	Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His	
95					100					105					110	
CTG	TGC	TGC	ATT	TCT	CTG	GAT	AGG	TAT	TAC	GCC	ATC	TGC	TGC	CAG	CCT	384
Leu	Cys	Cys	Ile	Ser	Leu	Asp	Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro	
				115					120					125		
TTG	GTC	TAT	AGG	AAC	AAG	ATG	ACC	CCT	CTG	CGC	ATC	GCA	TTA	ATG	CTG	432
Leu	Val	Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu	
			130					135					140			
GGA	GGC	TGC	TGG	GTC	ATC	CCC	ACG	TTT	ATT	TCT	TTT	CTC	CCT	ATA	ATG	480
Gly	Gly	Cys	Trp	Val	Ile	Pro	Thr	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met	
		145					150					155				
CAA	GGC	TGG	AAT	AAC	ATT	GGC	ATA	ATT	GAT	TTG	ATA	GAA	AAG	AGG	AAG	528
Gln	Gly	Trp	Asn	Asn	Ile	Gly	Ile	Ile	Asp	Leu	Ile	Glu	Lys	Arg	Lys	
	160					165					170					
TTC	AAC	CAG	AAC	TCT	AAC	TCT	ACG	TAC	TGT	GTC	TTC	ATG	GTC	AAC	AAG	576
Phe	Asn	Gln	Asn	Ser	Asn	Ser	Thr	Tyr	Cys	Val	Phe	Met	Val	Asn	Lys	
175					180					185					190	
CCC	TAC	GCC	ATC	ACC	TGC	TCT	GTG	GTG	GCC	TTC	TAC	ATC	CCA	TTT	CTC	624
Pro	Tyr	Ala	Ile	Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu	
				195					200					205		
CTC	ATG	GTG	CTG	GCC	TAT	TAC	CGC	ATC	TAT	GTC	ACA	GCT	AAG	GAG	CAT	672
Leu	Met	Val	Leu	Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His	
			210					215					220			
GCC	CAT	CAG	ATC	CAG	ATG	TTA	CAA	CGG	GCA	GGA	GCC	TCC	TCC	GAG	AGC	720
Ala	His	Gln	Ile	Gln	Met	Leu	Gln	Arg	Ala	Gly	Ala	Ser	Ser	Glu	Ser	
		225					230					235				
AGG	CCT	CAG	TCG	GCA	GAC	CAG	CAT	AGC	ACT	CAT	CGC	ATG	AGG	ACA	GAG	768

```
Arg  Pro  Gln  Ser  Ala  Asp  Gln  His  Ser  Thr  His  Arg  Met  Arg  Thr  Glu
     240                      245                      250

ACC  AAA  GCA  GCC  AAG  ACC  CTG  TGC  ATC  ATC  ATG  GGT  TGC  TTC  TGC  CTC      816
Thr  Lys  Ala  Ala  Lys  Thr  Leu  Cys  Ile  Ile  Met  Gly  Cys  Phe  Cys  Leu
255                      260                      265                      270

TGC  TGG  GCA  CCA  TTC  TTT  GTC  ACC  AAT  ATT  GTG  GAT  CCT  TTC  ATA  GAC      864
Cys  Trp  Ala  Pro  Phe  Phe  Val  Thr  Asn  Ile  Val  Asp  Pro  Phe  Ile  Asp
               275                                 280                 285

TAC  ACT  GTC  CCT  GGG  CAG  GTG  TGG  ACT  GCT  TTC  CTC  TGG  CTC  GGC  TAT      912
Tyr  Thr  Val  Pro  Gly  Gln  Val  Trp  Thr  Ala  Phe  Leu  Trp  Leu  Gly  Tyr
               290                      295                 300

ATC  AAT  TCC  GGG  TTG  AAC  CCT  TTT  CTC  TAC  GCC  TTC  TTG  AAT  AAG  TCT      960
Ile  Asn  Ser  Gly  Leu  Asn  Pro  Phe  Leu  Tyr  Ala  Phe  Leu  Asn  Lys  Ser
          305                      310                 315

TTT  AGA  CGT  GCC  TTC  CTC  ATC  ATC  CTC  TGC  TGT  GAT  GAT  GAG  CGC  TAC     1008
Phe  Arg  Arg  Ala  Phe  Leu  Ile  Ile  Leu  Cys  Cys  Asp  Asp  Glu  Arg  Tyr
     320                      325                 330

CGA  AGA  CCT  TCC  ATT  CTG  GGC  CAG  ACT  GTC  CCT  TGT  TCA  ACC  ACA  ACC     1056
Arg  Arg  Pro  Ser  Ile  Leu  Gly  Gln  Thr  Val  Pro  Cys  Ser  Thr  Thr  Thr
335                      340                 345                      350

ATT  AAT  GGA  TCC  ACA  CAT  GTA  CTA  AGG  GAT  GCA  GTG  GAG  TGT  GGT  GGC     1104
Ile  Asn  Gly  Ser  Thr  His  Val  Leu  Arg  Asp  Ala  Val  Glu  Cys  Gly  Gly
               355                      360                 365

CAG  TGG  GAG  AGT  CAG  TGT  CAC  CCG  CCA  GCA  ACT  TCT  CCT  TTG  GTG  GCT     1152
Gln  Trp  Glu  Ser  Gln  Cys  His  Pro  Pro  Ala  Thr  Ser  Pro  Leu  Val  Ala
               370                      375                 380

GCT  CAG  CCC  AGT  GAC  ACT  TAGGCCCCTG     GGACAATGAC     CCAGAAGACA          1200
Ala  Gln  Pro  Ser  Asp  Thr
          385

GCCATGCCTC     CGAAAGAGGG     CCAGGTCCTA     AGCTGCTGCT     TGTGCGCGAC     TGCACCCGGC     1260

ATTCTCTTCA     CCTGAGGCTT     TCCGTCCGCC     AGTGCAGGAA     CCCGGTGCTC     GCTGGG          1316


(2)  INFORMATION     FOR  SEQ  ID  NO:8:
```

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 388 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Asp	Lys	Leu	Asp	Ala	Asn	Val	Ser	Ser	Glu	Glu	Gly	Phe	Gly	Ser
1				5					10					15	
Val	Glu	Lys	Val	Val	Leu	Leu	Thr	Phe	Leu	Ser	Thr	Val	Ile	Leu	Met
			20					25					30		
Ala	Ile	Leu	Gly	Asn	Leu	Leu	Val	Met	Val	Ala	Val	Cys	Trp	Asp	Arg
		35					40					45			
Gln	Leu	Arg	Lys	Ile	Lys	Thr	Asn	Tyr	Phe	Ile	Val	Ser	Leu	Ala	Phe
	50					55					60				
Ala	Asp	Leu	Leu	Val	Ser	Val	Leu	Val	Met	Pro	Phe	Gly	Ala	Ile	Glu
65					70					75					80
Leu	Val	Gln	Asp	Ile	Trp	Ile	Tyr	Gly	Glu	Val	Phe	Cys	Leu	Val	Arg
				85					90					95	
Thr	Ser	Leu	Asp	Val	Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His	Leu	Cys
			100					105					110		
Cys	Ile	Ser	Leu	Asp	Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro	Leu	Val
		115					120					125			
Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu	Gly	Gly
	130					135					140				
Cys	Trp	Val	Ile	Pro	Thr	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met	Gln	Gly
145					150					155					160
Trp	Asn	Asn	Ile	Gly	Ile	Ile	Asp	Leu	Ile	Glu	Lys	Arg	Lys	Phe	Asn
				165					170					175	
Gln	Asn	Ser	Asn	Ser	Thr	Tyr	Cys	Val	Phe	Met	Val	Asn	Lys	Pro	Tyr
			180					185					190		

```
Ala Ile Thr Cys Ser Val Val Ala Phe Tyr Ile Pro Phe Leu Leu Met
          195                 200                 205

Val Leu Al  Tyr Tyr Arg Ile Tyr Val Thr Ala Lys Glu His Ala His
    210                 215                 220

Gln Ile Gln Met Leu Gln Arg Ala Gly Ala Ser Ser Glu Ser Arg Pro
225                 230                 235                 240

Gln Ser Ala Asp Gln His Ser Thr His Arg Met Arg Thr Glu Thr Lys
                245                 250                 255

Ala Ala Lys Thr Leu Cys Ile Ile Met Gly Cys Phe Cys Leu Cys Trp
          260                 265                 270

Ala Pro Phe Phe Val Thr Asn Ile Val Asp Pro Phe Ile Asp Tyr Thr
          275                 280                 285

Val Pro Gly Gln Val Trp Thr Ala Phe Leu Trp Leu Gly Tyr Ile Asn
    290                 295                 300

Ser Gly Leu Asn Pro Phe Leu Tyr Ala Phe Leu Asn Lys Ser Phe Arg
305                 310                 315                 320

Arg Ala Phe Leu Ile Ile Leu Cys Cys Asp Asp Glu Arg Tyr Arg Arg
                325                 330                 335

Pro Ser Ile Leu Gly Gln Thr Val Pro Cys Ser Thr Thr Thr Ile Asn
          340                 345                 350

Gly Ser Thr His Val Leu Arg Asp Ala Val Glu Cys Gly Gly Gln Trp
          355                 360                 365

Glu Ser Gln Cys His Pro Pro Ala Thr Ser Pro Leu Val Ala Ala Gln
    370                 375                 380

Pro Ser Asp Thr
385
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 14

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGGAATTCTG YGYAATHKCA CTGGAYMGST A 31

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 4

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 7

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 9..10

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 13

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATAAVARAA ARAGGDATRW ARAAAGC 27

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCAAAAGCAT GATTCCAGGG ACTCTGGGTC ATTGTGTATG GGCAA 45

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCTCAATCAG AAGCATGATT CCAGG 25

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTGGTCTATA GGAACAAGAT GACCC 25

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 792 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: Brain

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: HUMAN BRAIN
(B) CLONE: S10-87

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..783

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
TTG GTC TAT AGG AAC AAG ATG ACC CCT CTG CGC ATC GCA TTA ATG CTG   48
Leu Val Tyr Arg Asn Lys Met Thr Pro Leu Arg Ile Ala Leu Met Leu
 1               5                  10                  15

GGA GGC TGC TGG GTC ATC CCC ACG TTT ATT TCT TTT CTC CCT ATA ATG   96
Gly Gly Cys Trp Val Ile Pro Thr Phe Ile Ser Phe Leu Pro Ile Met
            20                  25                  30

CAA GGC TGG AAT AAC ATT GGC ATA ATT GAT TTG ATA GAA AAG AGG AAG  144
Gln Gly Trp Asn Asn Ile Gly Ile Ile Asp Leu Ile Glu Lys Arg Lys
        35                  40                  45

TTC AAC CAG AAC TCT AAC TCT ACG TAC TGT GTC TTC ATG GTC AAC AAG  192
Phe Asn Gln Asn Ser Asn Ser Thr Tyr Cys Val Phe Met Val Asn Lys
    50                  55                  60

CCC TAC GCC ATC ACC TGC TCT GTG GTG GCC TTC TAC ATC CCA TTT CTC  240
Pro Tyr Ala Ile Thr Cys Ser Val Val Ala Phe Tyr Ile Pro Phe Leu
65                  70                  75                  80

CTC ATG GTG CTG GCC TAT TAC CGC ATC TAT GTC ACA GCT AAG GAG CAT  288
Leu Met Val Leu Ala Tyr Tyr Arg Ile Tyr Val Thr Ala Lys Glu His
                85                  90                  95

GCC CAT CAG ATC CAG ATG TTA CAA CGG GCA GGA GCC TCC TCC GAG AGC  336
Ala His Gln Ile Gln Met Leu Gln Arg Ala Gly Ala Ser Ser Glu Ser
            100                 105                 110

AGG CCT CAG TCG GCA GAC CAG CAT AGC ACT CAT CGC ATG AGG ACA GAG  384
Arg Pro Gln Ser Ala Asp Gln His Ser Thr His Arg Met Arg Thr Glu
        115                 120                 125
```

```
ACC AAA GCA GCC AAG ACC CTG TGC ATC ATC ATG GGT TGC TTC TGC CTC          432
Thr Lys Ala Ala Lys Thr Leu Cys Ile Ile Met Gly Cys Phe Cys Leu
    130                 135                 140

TGC TGG GCA CCA TTC TTT GTC ACC AAT ATT GTG GAT CCT TTC ATA GAC          480
Cys Trp Ala Pro Phe Phe Val Thr Asn Ile Val Asp Pro Phe Ile Asp
145                 150                 155                 160

TAC ACT GTC CCT GGG CAG GTG TGG ACT GCT TTC CTC TGG CTC GGC TAT          528
Tyr Thr Val Pro Gly Gln Val Trp Thr Ala Phe Leu Trp Leu Gly Tyr
                165                 170                 175

ATC AAT TCC GGG TTG AAC CCT TTT CTC TAC GCC TTC TTG AAT AAG TCT          576
Ile Asn Ser Gly Leu Asn Pro Phe Leu Tyr Ala Phe Leu Asn Lys Ser
            180                 185                 190

TTT AGA CGT GCC TTC CTC ATC ATC CTC TGC TGT GAT GAT GAG CGC TAC          624
Phe Arg Arg Ala Phe Leu Ile Ile Leu Cys Cys Asp Asp Glu Arg Tyr
        195                 200                 205

CGA AGA CCT TCC ATT CTG GGC CAG ACT GTC CCT TGT TCA ACC ACA ACC          672
Arg Arg Pro Ser Ile Leu Gly Gln Thr Val Pro Cys Ser Thr Thr Thr
    210                 215                 220

ATT AAT GGA TCC ACA CAT GTA CTA AGG TAC ACC GTT CTG CAC AGG GGA          720
Ile Asn Gly Ser Thr His Val Leu Arg Tyr Thr Val Leu His Arg Gly
225                 230                 235                 240

CAT CAT CAG GAA CTC GAG AAA CTG CCC ATA CAC AAT GAC CCA GAA TCC          768
His His Gln Glu Leu Glu Lys Leu Pro Ile His Asn Asp Pro Glu Ser
                245                 250                 255

CTG GAA TCA TGC TTC TGATTGAGG                                            792
Leu Glu Ser Cys Phe
            260
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 261 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Leu Val Tyr Arg Asn Lys Met Thr Pro Leu Arg Ile Ala Leu Met Leu
1 5 10 15

Gly Gly Cys Trp Val Ile Pro Thr Phe Ile Ser Phe Leu Pro Ile Met
20 25 30

Gln Gly Trp Asn Asn Ile Gly Ile Ile Asp Leu Ile Glu Lys Arg Lys
35 40 45

Phe Asn Gln Asn Ser Asn Ser Thr Tyr Cys Val Phe Met Val Asn Lys
50 55 60

Pro Tyr Ala Ile Thr Cys Ser Val Val Ala Phe Tyr Ile Pro Phe Leu
65 70 75 80

Leu Met Val Leu Ala Tyr Tyr Arg Ile Tyr Val Thr Ala Lys Glu His
85 90 95

Ala His Gln Ile Gln Met Leu Gln Arg Ala Gly Ala Ser Ser Glu Ser
100 105 110

Arg Pro Gln Ser Ala Asp Gln His Ser Thr His Arg Met Arg Thr Glu
115 120 125

Thr Lys Ala Ala Lys Thr Leu Cys Ile Ile Met Gly Cys Phe Cys Leu
130 135 140

Cys Trp Ala Pro Phe Phe Val Thr Asn Ile Val Asp Pro Phe Ile Asp
145 150 155 160

Tyr Thr Val Pro Gly Gln Val Trp Thr Ala Phe Leu Trp Leu Gly Tyr
165 170 175

Ile Asn Ser Gly Leu Asn Pro Phe Leu Tyr Ala Phe Leu Asn Lys Ser
180 185 190

Phe Arg Arg Ala Phe Leu Ile Ile Leu Cys Cys Asp Asp Glu Arg Tyr
195 200 205

Arg Arg Pro Ser Ile Leu Gly Gln Thr Val Pro Cys Ser Thr Thr Thr
210 215 220

Ile Asn Gly Ser Thr His Val Leu Arg Tyr Thr Val Leu His Arg Gly

225					230					235					240
His	His	Gln	Glu	Leu	Glu	Lys	Leu	Pro	Ile	His	Asn	Asp	Pro	Glu	Ser
				245					250					255	
Leu	Glu	Ser	Cys	Phe											
			260												

What is claimed is:

1. An isolated nucleic acid molecule encoding a mammalian 5-HT_4 receptor.

2. A nucleic acid molecule of claim 1, wherein the nucleic acid molecule encodes a human 5-HT_4 receptor.

3. A nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a DNA molecule.

4. A DNA molecule of claim 3, wherein the DNA molecule is a cDNA molecule.

5. A DNA molecule of claim 3 wherein the DNA molecule is genomic DNA.

6. A nucleic acid molecule of claim 2, wherein the nucleic acid molecule is a DNA molecule.

7. An isolated DNA molecule encoding a mammalian 5-HT_4 receptor having the sequence H_2N-Y-X-COOH wherein Y is the amino acid sequence beginning at amino acid 1 and ending at amino acid 359 of Figure 1 (SEQ ID NO. 2) and wherein X is an amino acid sequence encoding the carboxy terminal region of the receptor.

8. An isolated nucleic acid molecule of claim 7, wherein X is the amino acid sequence beginning at amino acid 360 and ending at amino acid 387 of Figure 1 (SEQ ID NO. 2).

9. An isolated nucleic acid molecule of claim 7, wherein X is the amino acid sequence beginning at

amino acid 360 and ending at amino acid 406 of Figur 2 (SEQ ID NO. 4).

10. An isolated nucleic acid molecule of claim 7, wherein Y is encoded by the nucleotide sequence from nucleotide 101 to nucleotide 1177 of Figure 1 (SEQ ID NO. 1).

11. An isolated nucleic acid molecule of claim 8, wherein X is encoded by the nucleotide sequence from nucleotide 1178 to nucleotide to 1261 of Figure 1 (SEQ ID NO. 1).

12. An isolated nucleic acid molecule of claim 9, wherein X is encoded by the nucleotide sequence from nucleotide 1127 to nucleotide 1267 of Figure 2 (SEQ ID NO. 3).

13. A vector comprising a cDNA molecule of claim 4.

14. A plasmid vector of claim 13.

15. A vector of claim 13 adapted for expression in a bacterial cell which comprises regulatory elements necessary for expression of the cDNA encoding a 5-HT_4 receptor in the bacterial cell operatively linked to the cDNA encoding the 5-HT_4 receptor as to permit expression thereof.

16. A vector of claim 13 adapted for expression in a yeast cell which comprises the regulatory elements necessary for the expression of the cDNA encoding a 5-HT_4 receptor in the yeast cell operatively linked to the cDNA encoding the 5-HT_4 receptor as to permit expression thereof.

17. A vector of claim 13 adapted for expression in an

insect cell which comprises the regulatory elements necessary for expression of the cDNA encoding a 5-HT_4 receptor in the insect cell operatively linked to the cDNA encoding the 5-HT_4 receptor as to permit expression thereof.

18. A vector of claim 13 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the cDNA encoding a 5-HT_4 receptor in the mammalian cell operatively linked to the cDNA encoding the 5-HT_4 receptor as to permit expression thereof.

19. A plasmid of claim 14 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell operatively linked to the DNA encoding a 5-HT_4 receptor as to permit expression thereof.

20. A plasmid of claim 19 designated pcEXV-S10-87 (ATCC Accession No. 75390).

21. A plasmid of claim 19 designated pcEXV-S10-95 (ATCC Accession No. 75391).

22. A plasmid of claim 19 designated pBluescript-hS10 (ATCC Accession No. 75392).

23. A mammalian cell comprising the plasmid of claim 14.

24. A mammalian cell of claim 23, wherein the mammalian cell is an LM (tk-) cell.

25. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucle tides capable of

specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian 5-HT_4 receptor.

26. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT_4 receptor.

27. The nucleic acid probe of claim 25, wherein the nucleic acid is DNA.

28. The nucleic acid probe of claim 26, wherein the nucleic acid is DNA.

29. A mixture of nucleic acid probes in accordance with claim 25, such probes having sequences which differ from one another at predefined positions.

30. An antisense oligonucleotide having a sequence capable of specifically binding to a mRNA molecule encoding a mammalian 5-HT_4 receptor so as to prevent translation of the mRNA molecule.

31. An antisense oligonucleotide capable of specifically binding to a mRNA molecule encoding a human 5-HT_4 receptor so as to prevent translation of the mRNA molecule.

32. An antisense oligonucleotide of claim 30 comprising chemical analogs of nucleotides.

33. A mixture of antisense oligonucleotides according to claim 30, such oligonucleotides having sequences which differ from one another at

predefined positions.

34. A method for det cting expression of a mammalian 5-HT_4 receptor, which comprises obtaining RNA from cells or tissue, contacting the RNA so obtained with a nucleic acid probe of claim 25 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of the mammalian 5-HT_4 receptor, and thereby detecting the expression of the mammalian 5-HT_4 receptor.

35. A method for detecting expression of a human 5-HT_4 receptor, which comprises obtaining RNA from cells or tissue, contacting the RNA so obtained with a nucleic acid probe of claim 26 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of the human 5-HT_4 receptor, and thereby detecting the expression of the human 5-HT_4 receptor.

36. A method of detecting expression of a mammalian 5-HT_4 receptor in a cell or tissue by in situ hybridization, which comprises contacting the cell or tissue with a nucleic acid probe of claim 25 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of a mammalian 5-HT_4 receptor, and thereby detecting the expression of a mammalian 5-HT_4 receptor.

37. A method of detecting expression of a human 5-HT_4 receptor in a cell or tissue by in situ hybridization, which comprises contacting the cell

or tissue with a nucleic acid pr be of claim 26 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of a human 5-HT_4 receptor, and thereby detecting the expression of the human 5-HT_4 receptor.

38. A method of isolating from a gene library a gene encoding a receptor other than the 5-HT_4 receptor which comprises contacting the library under hybridizing conditions with a probe of claim 27 and isolating any gene to which the probe hybridizes.

39. A method of claim 38, which additionally comprises simultaneously contacting the DNA comprising the library under hybridizing conditions with a second nucleic acid probe comprising a sequence capable of hybridizing to a DNA sequence of the complementary strand of the DNA of the gene to which the first probe hybridizes, treating any gene sequence to which both probes hybridized so as to produce multiple copies of the gene sequence, isolating the amplified gene sequence and using the isolated gene sequence as a probe to isolate from a gene library the gene to which the amplified DNA sequence hybridizes.

40. The gene isolated by the method of claim 38 or 39.

41. A synthetic gene which comprises the isolated nucleic acid molecule of claim 1 and at least one regulatory element attached thereto so as to increase the number of RNA molecules transcribed from the synthetic g ne.

42. A synthetic gene which comprises the isolated nucleic acid molecule of claim 1 and at least one regulatory element attached thereto so as to decrease the number of RNA molecules transcribed from the synthetic gene.

43. An isolated mammalian 5-HT_4 receptor protein.

44. The receptor protein of claim 43, wherein the mammalian 5-HT_4 receptor protein is a human 5-HT_4 receptor.

45. A method of preparing a mammalian 5-HT_4 receptor of claim 43, which comprises inducing cells to express the mammalian 5-HT_4 receptor and recovering the mammalian 5-HT_4 receptor from the resulting cells.

46. A method of preparing a mammalian 5-HT_4 receptor protein of claim 43, which comprises inserting a nucleic acid molecule encoding the mammalian 5-HT_4 receptor in a suitable vector, inserting the resulting vector in suitable host cell and recovering the mammalian 5-HT_4 receptor produced by the resulting cell.

47. A method of preparing a human 5-HT_4 receptor of claim 44, which comprises inducing cells to express the human 5-HT_4 receptor and recovering the human 5-HT_4 receptor from the resulting cells.

48. A method of preparing a human 5-HT_4 receptor protein of claim 44, which comprises inserting a nucleic acid molecule encoding the human 5-HT_4 receptor in a suitable vector, inserting the resulting vector in suitable host cell and recovering the human 5-HT_4 receptor produced by

the resulting cell.

49. An antibody directed to a mammalian 5-HT_4 receptor or to a protein fragment of the mammalian 5-HT_4 receptor.

50. An antibody directed to a human 5-HT_4 receptor or to a protein fragment of the human 5-HT_4 receptor.

51. An antibody of claim 49, wherein the antibody is a monoclonal antibody.

52. An antibody of claim 50, wherein the antibody is a monoclonal antibody.

53. A monoclonal antibody of claim 51, wherein the antibody is directed to an epitope of a mammalian cell-surface 5-HT_4 receptor and having an amino acid sequence substantially the same as the amino acid sequence of a cell-surface epitope of the mammalian 5-HT_4 receptor.

54. A monoclonal antibody of claim 52, wherein the antibody is directed to an epitope of a human cell-surface 5-HT_4 receptor and having an amino acid sequence substantially the same as the amino acid sequence for a cell-surface epitope of the human 5-HT_4 receptor.

55. A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human 5-HT_4 receptor and a pharmaceutically acceptable carrier.

56. A pharmaceutical composition comprising an am unt of a substance effective to alleviate

abnormalities resulting from underexpression of a human 5-HT_4 receptor and a pharmaceutically acceptable carrier.

57. A pharmaceutical composition comprising an effective amount of an oligonucleotide of claim 31 effective to reduce expression of a human 5-HT_4 receptor by passing through a cell membrane and specifically binding with mRNA encoding a human 5-HT_4 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane.

58. A pharmaceutical composition claim 57, wherein the nucleotide is coupled to a substance which inactivates mRNA.

59. A pharmaceutical composition of claim 58, wherein the substance which inactivates the mRNA is a ribozyme.

60. A pharmaceutical composition of claim 58, wherein the pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane comprises a structure which binds to a transporter specific for a selected cell type and is thereby taken up by the cells of the selected cell type.

61. A pharmaceutical composition which comprises an amount of the antibody of claim 50 effective to block binding of naturally occurring substrates to a human 5-HT_4 receptor and a pharmaceutically acceptable carrier.

62. A transgenic nonhuman mammal which comprises a nucleic acid molecule of claim 1.

63. A transgenic nonhuman mammal whose genome comprises a nucleic acid molecule of claim 1 so placed as to be transcribed into antisense mRNA complementary to mRNA encoding a human 5-HT_4 receptor and which hybridizes to mRNA encoding a human 5-HT_4 receptor thereby reducing its translation.

64. The transgenic nonhuman mammal of claim 62, wherein the nucleic acid molecule further comprises an inducible promoter.

65. The transgenic nonhuman mammal of claim 62 or 64 wherein the nucleic molecule additionally comprises tissue specific regulatory elements.

66. The transgenic non-human mammal of 62 wherein the transgenic non-human mammal is a mouse.

67. A method for determining the physiological effects of varying the levels of expression of a human 5-HT_4 receptor which comprises producing a transgenic non-human mammal whose levels of expression of a human 5-HT_4 receptor can be varied by use of an inducible promoter.

68. A method for determining the physiological effects of expressing varying levels of a human 5-HT_4 receptor which comprises producing a panel of transgenic non-human mammals each expressing a different amount of a human 5-HT_4 receptor.

69. A method for determining whether a compound not known to be capable of specifically binding to a human 5-HT_4 receptor can specifically bind to the human 5-HT_4 receptor, which comprises contacting a mammalian cell comprising a plasmid adapted for

expression in a mammalian cell which plasmid furth r comprises DNA which express s a human 5-HT_4 receptor on the cell's surface with th compound under conditions permitting binding of ligands known to bind to a human 5-HT_4 receptor, detecting the presence of any compound bound to the human 5-HT_4 receptor, the presence of bound compound indicating that the compound is capable of specifically binding to the human 5-HT_4 receptor.

70. The method of claim 70, wherein the mammalian cell is a non-neuronal cell.

71. A method of screening compounds to identify drugs which interact with, and specifically bind to, a human 5-HT_4 receptor on the surface of a cell, which comprises contacting a mammalian cell which comprises a plasmid adapted for expression in a mammalian cell which plasmid further comprises DNA which expresses a human 5-HT_4 receptor on the cell's surface with a plurality of compounds, determining those compounds which bind to the human 5-HT_4 receptor expressed on the cell surface of the mammalian cell, and thereby identifying compounds which interact with, and specifically bind to, the human 5-HT_4 receptor.

72. The method of claim 71, wherein the mammalian cell is a non-neuronal cell.

73. A method for determining whether a compound not known to be capable of specifically binding to a human 5-HT_4 receptor can specifically bind to a human 5-HT_4 receptor, which comprises preparing a cell extract from mammalian cells, which comprise a plasmid adapted for expression in a mammal,

which plasmid further comprises DNA which expresses a human 5-HT_4 receptor on the cell's surface, isolating a membrane fraction from the cell extract, incubating the compound with the membrane fraction under conditions permitting binding of ligands known to bind to the human 5-HT_4 receptor, detecting the presence of any bound compound, and thereby determining whether the compound is capable of specifically binding to the human 5-HT_4 receptor.

74. The method of claim 73, wherein the mammalian cell is a non-neuronal cell.

75. A method for screening compounds to identify drugs that interact with, and specifically bind to, a human 5-HT_4 receptor, which comprises preparing a cell extract from mammalian cells, which comprise a plasmid adapted for expression in a mammalian cell which plasmid further comprises DNA which expresses a human 5-HT_4 receptor on the cell's surface, isolating a membrane fraction from the cell extract, incubating the membrane fraction with a plurality of compounds, determining those compounds which interact with and bind to the human 5-HT_4 receptor, and thereby identifying compounds which interact with, and specifically bind to, the human 5-HT_4 receptor.

76. The method of claim 75, wherein the mammalian cell is a non-neuronal cell.

77. A method for identifying a compound which is not known to be capable of binding to the human 5-HT_4 receptor activates the human 5-HT_4 receptor on the surface of a mammalian cell or pr vents a ligand which does so, which comprises contacting the

mammalian cell which c ll comprises a plasmid adapted for expression in the mammalian cell such plasmid further comprising DNA which expresses the human 5-HT_4 receptor on the cell surface of the mammalian cell with the compound under conditions permitting activation of blockade of a functional response, determining whether the compound activates the human 5-HT_4 receptor or prevents a ligand which does so, and thereby identifying the compound as a compound which interacts with, and activates the human 5-HT_4 receptor or prevents the activation of the human 5-HT_4 receptor by a ligand which does so.

78. The method of claim 77, wherein the mammalian cell is a non-neuronal cell comprising the cellular components necessary to produce a second messenger and wherein the determination of whether the compound activates of blocks the activation of the human 5-HT_4 comprises detecting the change in the concentration of the second messenger.

79. The method of claim 78, wherein the second messenger is cyclic AMP (cAMP).

80. The method of claim 78, wherein the non-neuronal cell is a COS-7 cell.

81. A method of claim 78, wherein the second messenger is an inositol phosphate metabolite.

82. The method of claim 78, wherein the second messenger is intracellular calcium.

83. A compound identified by the method of claim 69, 73 or 77.

84. A pharmaceutical composition of a drug identified by the method of claim 71 or 75.

85. A method for detecting the presence of a human 5-HT_4 receptor on the surface of a cell, which comprises contacting the cell with an antibody of claim 50, under conditions that permit binding of the antibody to the receptor, detecting the presence of any of the antibody bound to the cell, and thereby the presence of a human 5-HT_4 receptor on the surface of the cell.

86. A method for treating an abnormal condition related to an excess of activity of a human 5-HT_4 receptor, which comprises administering a patient an amount of a pharmaceutical composition of claim 84, effective to reduce 5-HT_4 activity as a result of naturally occurring substrate binding to and activating the 5-HT_4 receptor.

87. The method of treating abnormalities which are alleviated by an increase in the activity of a 5-HT_4 receptor, which comprises administering a patient an amount of a pharmaceutical composition of claim 84, effective to increase the activity of the 5-HT_4 receptor thereby alleviating abnormalities resulting from abnormally low receptor activity.

88. A method for diagnosing a predisposition to a disorder associated with the expression of a human 5-HT_4 receptor allele which comprises:

a. obtaining DNA from subjects suffering from a disorder;

b. performing a restriction digest of the DNA

with a panel of restriction enzymes;

c. electrophoretically s parating the resulting DNA fragments on a sizing gel;

d. contacting the gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human 5-HT_4 receptor and labelled with a detectable marker;

e. detecting the labelled bands which have hybridized to the DNA encoding 5-HT_4 receptor, labelled with the detectable marker to create a unique band pattern specific to the DNA of subjects suffering with the disorder;

f. preparing DNA for diagnosis by steps a-e;

g. comparing the unique band pattern specific to the DNA of patients suffering from the disorder from step e and DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

89. The method of claim 88, wherein a disorder is associated with the expression of a specific human 5-HT_4 receptor allele is diagnosed.

90. A method of identifying a substance capable of alleviating the abnormalities resulting from overexpression of a human 5-HT_4 receptor which comprises administering a substance to the transgenic non-human mammal of claim 67 or 68, and determining whether the substance alleviates the

physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of the human 5-HT_4 receptor.

91. A method of identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human 5-HT_4 receptor, which comprises administering a substance to the transgenic mammal of claim 62, and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of the human 5-HT_4 receptor.

92. A method of treating abnormalities in a subject, wherein the abnormality is alleviated by the reduced expression of a human 5-HT_4 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 55, 57, 83 or 84 effective to reduce expression of the human 5-HT_4 receptor.

93. A method of treating abnormalities resulting from underexpression of a human 5-HT_4 receptor which comprises administering to a subject an amount of a pharmaceutical composition of claim 56, 83 or 84 effective to alleviate abnormalities resulting from underexpression of the human 5-HT_4 receptor.

FIGURE 1-1

FIGURE 1-1, 1/32
FIGURE 1-2, 2/32

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1   AGCCTTGCCGAGCCTGGCTTGGTTGGAAGGAGGAGGATGCTCTGCGTGCCCAGGGTCCTG   60
61  TGGGCACTGACATCCAACGTACTCATGCCCATTTCCTGTAATGGACAGACTTGATGCTAA   120
                                          M  D  R  L  D  A  N     7
121 TGTGAGTTCCAACGAGGGTTTCGGGTCTGTGGAGAAGGTCGTACTGCTCACGTTCTTCGC   180
8    V  S  S  N  E  G  F  G  S  V  E  K  V  V  L  L  T  F  F  A   27
181 AATGGTTATCCTGATGGCCATCCTGGGCAACCTGCTGGTGATGGTTGCTGTGTGCAGGGA   240
28   M  V  I  L  M  A  I  L  G  N  L  L  V  M  V  A  V  C  R  D   47
241 CAGGCAGCTCAGGAAAATAAAAACCAATTATTTCATTGTGTCTCTTGCCTTTGCTGATCT   300
48   R  Q  L  R  K  I  K  T  N  Y  F  I  V  S  L  A  F  A  D  L   67
301 GCTGGTTTCGGTGCTGGTGAATGCCTTCGGTGCCATTGAGTTGGTCCAAGACATCTGGTT   360
68   L  V  S  V  L  V  N  A  F  G  A  I  E  L  V  Q  D  I  W  F   87
361 TTATGGGGagatgttttgcctggtccggacctctctggatgtcctactcaccacagcatc   420
88   Y  G  E  M  F  C  L  V  R  T  S  L  D  V  L  L  T  T  A  S   107
421 aatttttcacctctgctgcatttcccTGGATAGGTATTATGCCATCTGCTGTCAACCTTT   480
108  I  F  H  L  C  C  I  S  L  D  R  Y  Y  A  I  C  C  Q  P  L   127
481 GGTTTATAGAAACAAGATGACCCCTCTACGCATCGCATTAATGCTGGGAGGCTGCTGGGT   540
128  V  Y  R  N  K  M  T  P  L  R  I  A  L  M  L  G  G  C  W  V   147
541 CATTCCCATGTTTATATCTTTTCTCCCCATAATGCAAGGCTGGAACAACATCGGCATAGT   600
148  I  P  M  F  I  S  F  L  P  I  M  Q  G  W  N  N  I  G  I  V   167
601 TGATGTGATAGAGAAAAGGAAATTCAACCACAACTCTAACTCTACATTCTGTGTCTTCAT   660
168  D  V  I  E  K  R  K  F  N  H  N  S  N  S  T  F  C  V  F  M   187
661 GGTCAACAAGCCCTATGCCATCACCTGCTCTGTGGTGGCCTTCTACATCCCGTTTCTCCT   720
188  V  N  K  P  Y  A  I  T  C  S  V  V  A  F  Y  I  P  F  L  L   207
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FIGURE 1-2

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721  CATGGTGCTGGCCTATTACCGTATCTATGTCACTGCTAAGGAGCATGCCCAGCAGATCCA  780
208   M  V  L  A  Y  Y  R  I  Y  V  T  A  K  E  H  A  Q  Q  I  Q   227
781  GATGTTACAACGGGCAGGAGCCACCTCTGAAAGCAGGCCCCAGACAGCTGACCAGCACAG  840
228   M  L  Q  R  A  G  A  T  S  E  S  R  P  Q  T  A  D  Q  H  S   247
841  CACACATCGCATGCGGACAGAGACCAAAGCAGCCAAGACTTTATGTGTCATCATGGGCTG  900
248   T  H  R  M  R  T  E  T  K  A  A  K  T  L  C  V  I  M  G  C   267
901  CTTCTGTTTCTGCTGGGCCCCCTTCTTTGTCACCAATATTGTGGACCCTTTCATAGACTA  960
268   F  C  F  C  W  A  P  F  F  V  T  N  I  V  D  P  F  I  D  Y   287
961  CACTGTGCCCGAGAAGGTGTGGACTGCTTTCCTCTGGCTTGGCTATATCAATTCAGGGTT  1020
288   T  V  P  E  K  V  W  T  A  F  L  W  L  G  Y  I  N  S  G  L   307
1021 GAACCCTTTTCTCTATGCCTTCTTGAATAAGTCTTTCAGACGTGCCTTCCTTATCATCCT  1080
308   N  P  F  L  Y  A  F  L  N  K  S  F  R  R  A  F  L  I  I  L   327
1081 CTGCTGTGATGATGAGCGCTACAAAAGACCCCCCATTCTGGGCCAGACTGTCCCCTGTTC  1140
328   C  C  D  D  E  R  Y  K  R  P  P  I  L  G  Q  T  V  P  C  S   347
1141 AACCACAACCATTAATGGATCCACTCATGTGCTAAGGTATACAGTTTTGCATAGTGGTCA  1200
348   T  T  T  I  N  G  S  T  H  V  L  R  Y  T  V  L  H  S  G  Q   367
1201 ACACCAGGAACTGGAGAAGTTGCCCATACACAATGACCCAGAGTCCCTGGAATCATGCTT  1260
368   H  Q  E  L  E  K  L  P  I  H  N  D  P  E  S  L  E  S  C  F   387
1261 TTGATTGAAGACGTGGCTTGCCTTTAGCGGTTCATCCCATCTGTGTCTGCATGAACAGGT  1320
                              *
1321 TACTATGGAATCACTCCTGACTCTGGGCATCACCAGTGAAGCATGAGCATGGTGAGGCAG  1380
1381 GGTCCGGTGAAGGTGCACAGAGGACAGCATTGAGTGGGACCTGAACCCAGCACATTAAGG  1440
1441 ATTTCAGAACCGTGTGGGGATTTGAGATGTCATCAGACCCAGTGTCTTACCCAGAGCCCA  1500
1501 ACTGGCACCTCCCATTCCACGCTGACATGTGGTCAGTCTTTGCTCACACCTCTCCAGGGG  1560
1561 CAGGAGCTGACTACCTCCTAATGTGGTGGGGAGCTCTTAATTGTGTGGAAGTTCAGTCAT  1620
1621 TCATTGGTGGACAGTCTCGCTG  1642
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FIGURE 2-1

FIGURE 2-1, 3/32
FIGURE 2-2, 4/32

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   1  CAGCGCACGAGCAGCGGCAGAAGCAGGGCTCTGCGCTAAAGGTGGCCGCAGCGATCTGCC   60
  61  AGGGTGCCGCTCTCCTTAGCCTTNCGCAGCCGCGTGGGGCTGGGAGTGCokGCCACGAGA  120
 121  TCAGAACCCCAGGAGCCCACGTGTGAAGGGTCCTGTGGGCACTGACATCCAACGTACTCA  180
 181  TGCCCATTTCCTGTAATGGACAGACTTGATGCTAATGTGAGTTCCAACGAGGGTTTCGGG  240
                M  D  R  L  D  A  N  V  S  S  N  E  G  F  G          15
 241  TCTGTGGAGAAGGTCGTACTGCTCACGTTCTTCGCAATGGTTATCCTGATGGCCATCCTG  300
  16  S  V  E  K  V  V  L  L  T  F  F  A  M  V  I  L  M  A  I  L    35
 301  GGCAACCTGCTGGTGATGGTTGCTGTGTGCAGGGACAGGCAGCTCAGGAAAATAAAAACC  360
  36  G  N  L  L  V  M  V  A  V  C  R  D  R  Q  L  R  K  I  K  T    55
 361  AATTATTTCATTGTGTCTCTTGCCTTTGCTGATCTGCTGGTTTCGGTGCTGGTGAATGCC  420
  56  N  Y  F  I  V  S  L  A  F  A  D  L  L  V  S  V  L  V  N  A    75
 421  TTCGGTGCCATTGAGTTGGTCCAAGACATCTGGTTTTATGGGGAGATGTTTTGCCTGGTC  480
  76  F  G  A  I  E  L  V  Q  D  I  W  F  Y  G  E  M  F  C  L  V    95
 481  CGGACCTCTCTGGATGTCCTACTCACCACAGCATCAATTTTTCACCTCTGCTGCCTTTCC  540
  96  R  T  S  L  D  V  L  L  T  T  A  S  I  F  H  L  C  C  I  S   115
 541  CTGGATAGGTATTATGCCATCTGCTGTCAACCTTTGGTTTATAGAAACAAGATGACCCCT  600
 116  L  D  R  Y  Y  A  I  C  C  Q  P  L  V  Y  R  N  K  M  T  P   135
 601  CTACGCATCGCATTAATGCTGGGAGGCTGCTGGGTCATTCCCATGTTTATATCTTTTCTC  660
 136  L  R  I  A  L  M  L  G  G  C  W  V  I  P  M  F  I  S  F  L   155
 661  CCCATAATGCAAGGCTGGAACAACATCGGCATAGTTGATGTGATAGAGAAAAGGAAATTC  720
 156  P  I  M  Q  G  W  N  N  I  G  I  V  D  V  I  E  K  R  K  F   175
 721  AACCACAACTCTAACTCTACATTCTGTGTCTTCATGGTCAACAAGCCCTATGCCATCACC  780
 176  N  H  N  S  N  S  T  F  C  V  F  M  V  N  K  P  Y  A  I  T   195
 781  TGCTCTGTGGTGGCCTTCTACATCCCGTTTCTCCTCATGGTGCTGGCCTATTACCGTATC  840
 196  C  S  V  V  A  F  Y  I  P  F  L  L  M  V  L  A  Y  Y  R  I   215
```

FIGURE 2-2

```
841  TATGTCACTGCTAAGGAGCATGCCCAGCAGATCCAGATGTTACAACGGGCAGGAGCCACC  900
216  Y  V  T  A  K  E  H  A  Q  Q  I  Q  M  L  Q  R  A  G  A  T    235
901  TCTGAAAGCAGGCCCCAGACAGCTGACCAGCACAGCACACATCGCATGCGGACAGAGACC  960
236  S  E  S  R  P  Q  T  A  D  Q  H  S  T  H  R  M  R  T  E  T    255
961  AAAGCAGCCAAGACTTTATGTGTCATCATGGGCTGCTTCTGTTTCTGCTGGGCCCCCTTC  1020
256  K  A  A  K  T  L  C  V  I  M  G  C  F  C  F  C  W  A  P  F    275
1021 TTTGTCACCAATATTGTGGACCCTTTCATAGACTACACTGTGCCCGAGAAGGTGTGGACT  1080
276  F  V  T  N  I  V  D  P  F  I  D  Y  T  V  P  E  K  V  W  T    295
1081 GCTTTCCTCTGGCTTGGCTATATCAATTCAGGGTTGAACCCTTTTCTCTATGCCTTCTTG  1140
296  A  F  L  W  L  G  Y  I  N  S  G  L  N  P  F  L  Y  A  F  L    315
1141 AATAAGTCTTTCAGACGTGCCTTCCTTATCATCCTCTGCTGTGATGATGAGCGCTACAAA  1200
316  N  K  S  F  R  R  A  F  L  I  I  L  C  C  D  D  E  R  Y  K    335
1201 AGACCCCCCATTCTGGGCCAGACTGTCCCCTGTTCAACCACAACCATTAATGGATCCACT  1260
336  R  P  P  I  L  G  Q  T  V  P  C  S  T  T  T  I  N  G  S  T    355
1261 CATGTGCTAAGGGATACAGTGGAATGTGGTGGCCAATGGGAGAGTCGGTGTCACCTCACA  1320
356  H  V  L  R  D  T  V  E  C  G  G  Q  W  E  S  R  C  H  L  T    375
1321 GCAACTTCTCCTTTGGTGGCTGCTCAGCCAGTGATACGTAGGCCCCAGGACAATGACCTA  1380
376  A  T  S  P  L  V  A  A  Q  P  V  I  R  R  P  Q  D  N  D  L    395
1381 GAAGACAGCTGTAGCTTGAAAAGAAGCCAGTCCTAAGCTGCTACTTCGGTGTATGTGGCT  1440
396  E  D  S  C  S  L  K  R  S  Q  S  *                           406
1441 GCCCCTGGCACTTTGTTCTCCAAGGCTTTCCAAGAGCATGAGGCAATCCACCCTGGACTT  1500
1501 CCCGCCACGATTCTAGCAGGCGGTATTAGAGGAAGTCAGGGGAGAGAAGGGCTTCCTCCT  1560
1561 TAGCTTTCTGTTTCTCAACATTTTCTCTTCCTGGAGTCTCCACTCTTGCTTGGTGGTCTC  1620
1621 TGAAGTCCACGACCCAGTCCCCTTTTGCTGTCTCCAGTCTGTCTTGTAAATGTTTACCGT  1680
1681 GTTCGATTTTCAGTTTCCAAACATGCCTTCTTTGAAGTGTCATCTTACGATACTGTCAAA  1740
1741 ACATGTGCCTGTCTTGATCACACTTCTT  1768
```

FIGURE 3

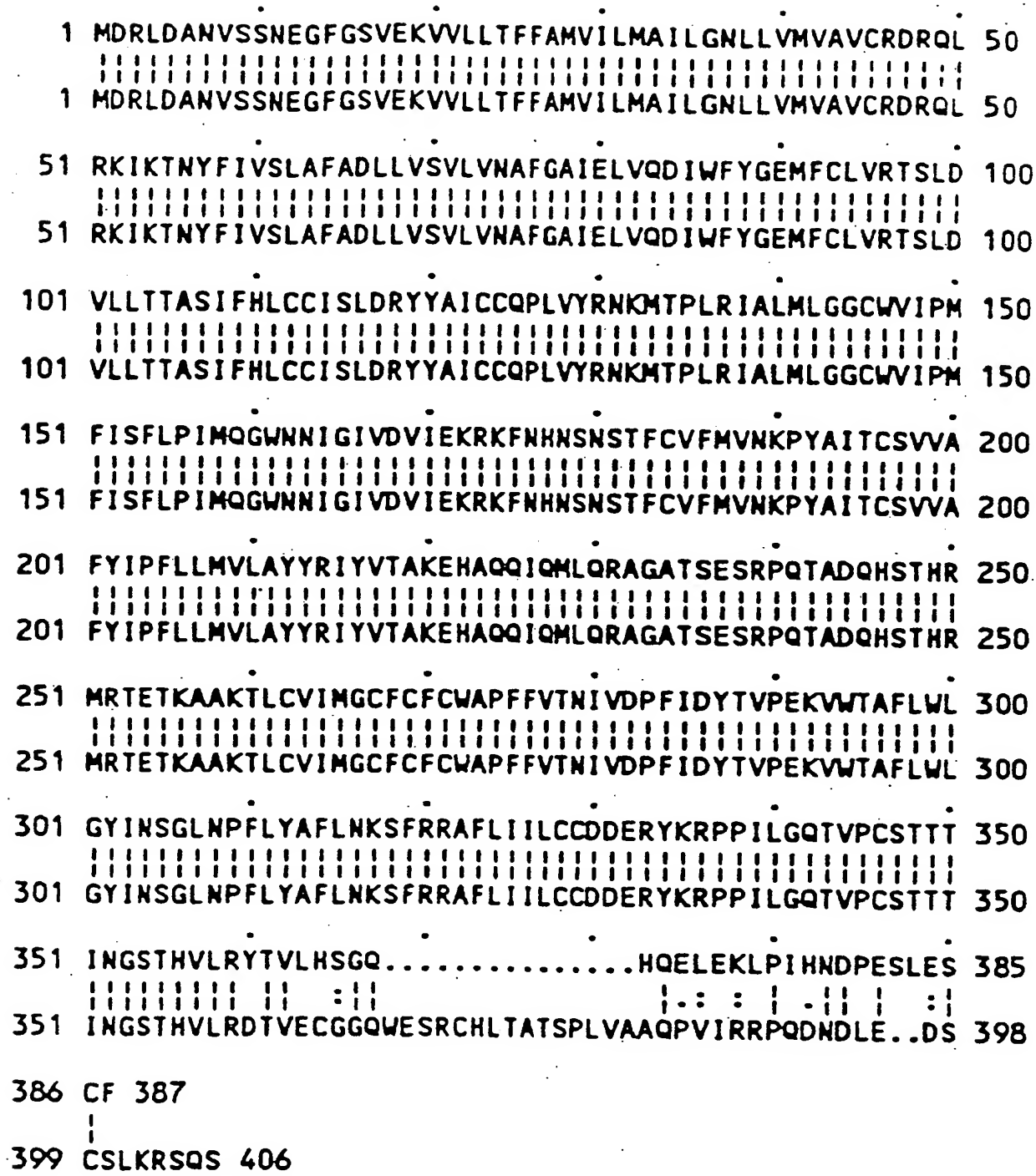

FIGURE 4-1

FIGURE 4-1, 6/32
FIGURE 4-2, 7/32
FIGURE 4-3, 8/32

```
S10-95                                                                                   0
S10-87                                                                                   0
Hp78        M M D V N S S G R P D L Y G H L R S F L L P E V G R G L P D L S P D G G A D P V A G S W A P H L L S   50
5-HT2 Rat                                  M E I L C E D N I S L S S I P N S L M Q L G D G P R L Y H N D F N S R   35
5-HT1C Rat                                                   M V N L G N A V R S L L M H L I G L   18
Hist2 Dog                                                                                0

                                                                                 ___________________
S10-95                            M D R L D A N V S S N E G F G S V E . . . . . . . . . . K V V L L T F F A M   28
S10-87                            M D R L D A N V S S N E G F G S V E . . . . . . . . . . K V V L L T F F A M   28
Hp78        E V T A S P A P T W D A P P D N A S G C G E Q I N Y Q R V E . . . . . . . . . . K V V I G S I L T L   90
5-HT2 Rat   D A N T S E A S N W T I D A E N R T N L S C E G Y L P P T C L S I L H L Q E . . K N W S A L L T T V   83
5-HT1C Rat  L V W Q F D I S I S P V A A . . . . . I V T D T F N S S D G G R L F Q F P D G V Q N W P A L S I V V   63
Hist2 Dog                           M I S N G T G S S F C L D S P P C . . . . . . . . . . R I T V S V V L T V   27

 ___ I ______________                                     ______________ II ______________
S10-95      V I L M A I L G N L L V M V A V C R D R Q L R K I K T N Y F I V S L A F A D L L V S V L V M A F Q A   78
S10-87      V I L M A I L G N L L V M V A V C R D R Q L R K I K T N Y F I V S L A F A D L L V S V L V M A F G A   78
Hp78        I T L L T I A Q N C L V V I S V C F V K K L R Q . P S N Y L I V S L A L A D L S V A V A V M P F V S  139
5-HT2 Rat   V I I L T I A G N I L V I M A V S L E K K L Q N . A T N Y F L M S L A I A D M L L G F L V M P V S M  132
5-HT1C Rat  I I I M T I G G N I L V I M A V S M E K K L H N . A Y N Y F L M S L A I A D M L V G L L V M P L S L  112
Hist2 Dog   L I L I T I A Q N V V V C L A V G L N R R L R S L . T N C F I V S L A I T D L L L G L L V L P F S A   76
```

FIGURE 4-2

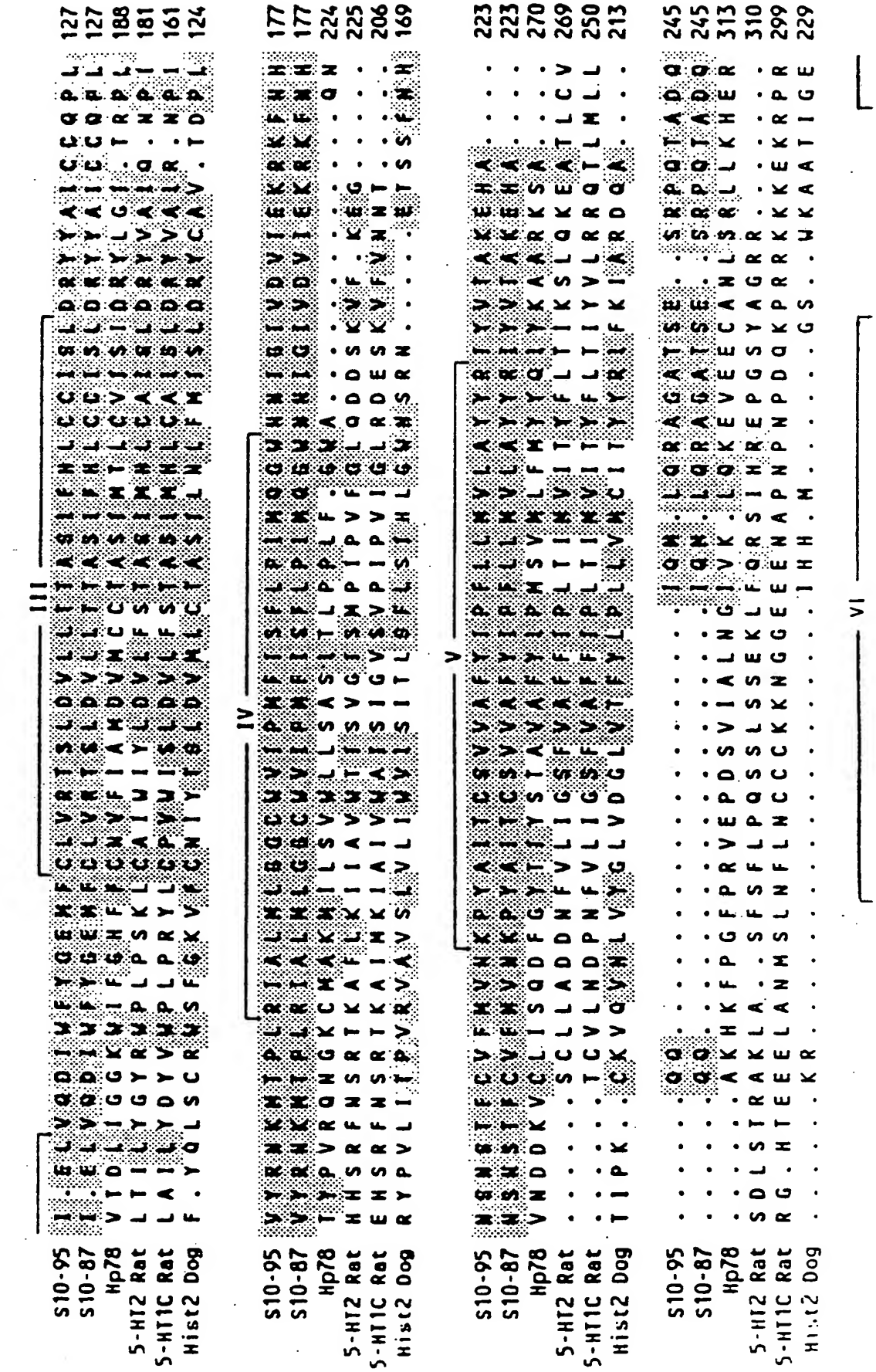

FIGURE 4-3

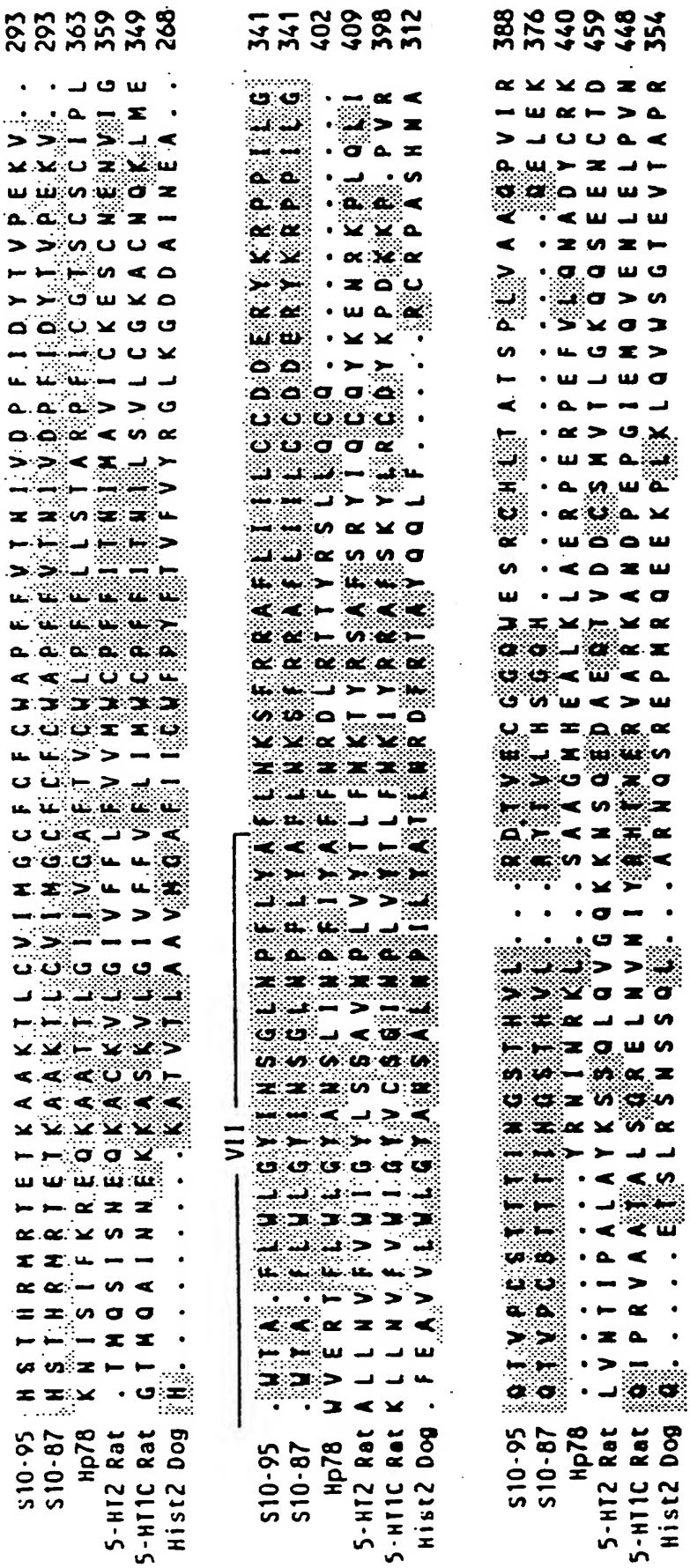
VII
S10-95 H S T H R M R T E T K A A K T L C V I M G C F C F C W A P F F V T N I V D P F I D Y T V P E K V . . 293
S10-87 H S T H R M R T E T K A A K T L C V I M G C F C F C W A P F F V T N I V D P F I D Y T V P E K V . . 293
Hp78 K N I S I F K R E Q K A A T T L G I I V G A F T V C W L P F F L L S T A R P F I C G T S C S C I P L 363
5-HT2 Rat . T M Q S I S N E Q K A C K V L G I V F F L F V V H W C P F F I T N I M A V I C K E S C N E N V I G 359
5-HT1C Rat G T M Q A I N N E K K A S K V L G I V F F V F L I H W C P F F I T N I L S V L C G K A C N Q K L M E 349
Hist2 Dog H . . . . . K A T V T L A A V N P Q A F I I C W F P Y F T V F V Y R G L K G D D A I N E A . . 268
S10-95 . W T A . F L W L G Y I H S G L N P F L Y A F L N K S F R R A F L I I L C C D E R Y K R P P I L G 341
S10-87 . W T A . F L W L G Y I H S G L N P P L Y A F L N K S F R R A F L I I L C C D D E R Y K R P P I L G 341
Hp78 W V E R T F L W L G Y A H S L I N P F I Y A F F N R D L R T T Y R S L L Q C Q 402
5-HT2 Rat A L L N V F V W I G Y L S S A V N P L V Y T L F N K T Y R S A F S R Y I Q C Q Y K E N R K P L Q L I 409
5-HT1C Rat K L L N V F V W I G Y V C S G I N P L V Y T L F N K I Y R R A F S K Y L R C D Y K P D K K P . P V R 398
Hist2 Dog . F E A V V L W L G Y A H S A L N P I L Y A T L N R D T R T A Y Q Q L F . . . . . R C R P A S H M A 312
S10-95 Q I V P C S T T T I N G S T H V L . . . R D T V E C G G Q W E S R C H L T A T S P L V A A Q P V I R 388
S10-87 Q T V P C S T T T I H Q S T H V L . . . A Y T Y V L H S G Q H . . . . . Q E L E K 376
Hp78 . . . . . Y R W I N R K L . . . S A A G H H E A L K L A E R P E P E R P E F V L Q N A D Y C R K 440
5-HT2 Rat L V N T I P A L A Y K S S Q L Q V G Q K K N S Q E D A E Q T V D D C S M V T L G K Q S E E N C T D 459
5-HT1C Rat Q I P R V A A T A L S Q R E L M V N I Y R H T H E R V A R K A N D P E P G I E M Q V E N L E L P V N 448
Hist2 Dog Q . . . . . E T S L R S N S S Q L . . . A R N Q S R E P M R Q E E K P L K L Q V W S G T E V T A P R 354
S10-95 R P Q D W D L E D Q C S L K R S Q S . 406
S10-87 L P I H N D P G S L E S C F 387
Hp78 K G H Q S 445
5-HT2 Rat N I E T V N E K V S C V 471
5-HT1C Rat P S N V V S E R I S S V 460
Hist2 Dog G A T D R 359

FIGURE 5

```
574  TTGGTCTATAGGAACAAGATGACCCCTCTGCGCATCGCATTAATGCTGGGAGGCTGCTGG   633
127  L  V  Y  R  N  K  M  T  P  L  R  I  A  L  M  L  G  G  C  W    146
634  GTCATCCCCACGTTTATTTCTTTTCTCCCTATAATGCAAGGCTGGAATAACATTGGCATA   693
147  V  I  P  T  F  I  S  F  L  P  I  M  Q  G  W  N  N  I  G  I    166
694  ATTGATTTGATAGAAAAGAGGAAGTTCAACCAGAACTCTAACTCTACGTACTGTGTCTTC   753
167  I  D  L  I  E  K  R  K  F  N  Q  N  S  N  S  T  Y  C  V  F    186
754  ATGGTCAACAAGCCCTACGCCATCACCTGCTCTGTGGTGGCCTTCTACATCCCATTTCTC   813
187  M  V  N  K  P  Y  A  I  T  C  S  V  V  A  F  Y  I  P  F  L    206
814  CTCATGGTGCTGGCCTATTACCGCATCTATGTCACAGCTAAGGAGCATGCCCATCAGATC   873
207  L  M  V  L  A  Y  Y  R  I  Y  V  T  A  K  E  H  A  H  Q  I    226
874  CAGATGTTACAACGGGCAGGAGCCTCCTCCGAGAGCAGGCCTCAGTCGGCAGACCAGCAT   933
227  Q  M  L  Q  R  A  G  A  S  S  E  S  R  P  Q  S  A  D  Q  H    246
934  AGCACTCATCCGATGAGGACAGAGACCAAAGCAGCCAAGACCCTGTGCATCATCATGGGT   993
247  S  T  H  P  M  R  T  E  T  K  A  A  K  T  L  C  I  I  M  G    266
994  TGCTTCTGCCTCTGCTGGGCACCATTCTTTGTCACCAATATTGTGGATCCTTTCATAGAC   1053
267  C  F  C  L  C  W  A  P  F  F  V  T  N  I  V  D  P  F  I  D    286
1054 TACACTGTCCCTGGGCAGGTGTGGACTGCTTTCCTCTGGCTCGGCTATATCAATTC   1109
287  Y  T  V  P  G  Q  V  W  T  A  F  L  W  L  G  Y  I  N        304
```

FIGURE 6

```
574  TTGGTCTATAGGAACAAGATGACCCCTCTGCGCATCGCATTAATGCTGGGAGGCTGCTGG   633
          T     A                    A
634  GTCATCCCCACGTTTATTTCTTTTCTCCCTATAATGCAAGGCTGGAATAACATTGGCATA   693
          T    T      A              C                C     C
694  ATTGATTTGATAGAAAAGAGGAAGTTCAACCAGAACTCTAACTCTACGTACTGTGTCTTC   753
     G     G        G  A     A        C                A T
754  ATGGTCAACAAGCCCTACGCCATCACCTGCTCTGTGGTGGCCTTCTACATCCCATTTCTC   813
                       T                                   G
814  CTCATGGTGCTGGCCTATTACCGCATCTATGTCACAGCTAAGGAGCATGCCCATCAGATC   873
                                 T          T                G
874  CAGATGTTACAACGGGCAGGAGCCTCCTCCGAGAGCAGGCCTCAGTCGGCAGACCAGCAT   933
                                   A    T  A        C   A A  T        C
934  AGCACTCATCCGATGAGGACAGAGACCAAAGCAGCCAAGACCCTGTGCATCATCATGGGT   993
          A    GC   C                                  TT A  TG        C
994  TGCTTCTGCCTCTGCTGGGCACCATTCTTTGTCACCAATATTGTGGATCCTTTCATAGAC   1053
               TT          C  C                            C
1054  TACACTGTCCCTGGGCAGGTGTGGACTGCTTTCCTCTGGCTCGGCTATATCAATTC     1109
               G  C A A                              T
```

FIGURE 7

```
127  LVYRNKMTPLRIALMLGGCWVIPTFISFLPIMQGWNNIGIIDLIEKRKFNQNSNSTYCVF  186
                    M                 V V       H     F
187  MVNKPYAITCSVVAFYIPFLLMVLAYYRIYVTAKEHAHQIQMLQRAGASSESRPQSADQH  246
                                       Q         T     T
247  STHPMRTETKAAKTLCIIMGCFCLCWAPFFVTNIVDPFIDYTVPGQVWTAFLWLGYIN  304
          R         V       F                       EK
```

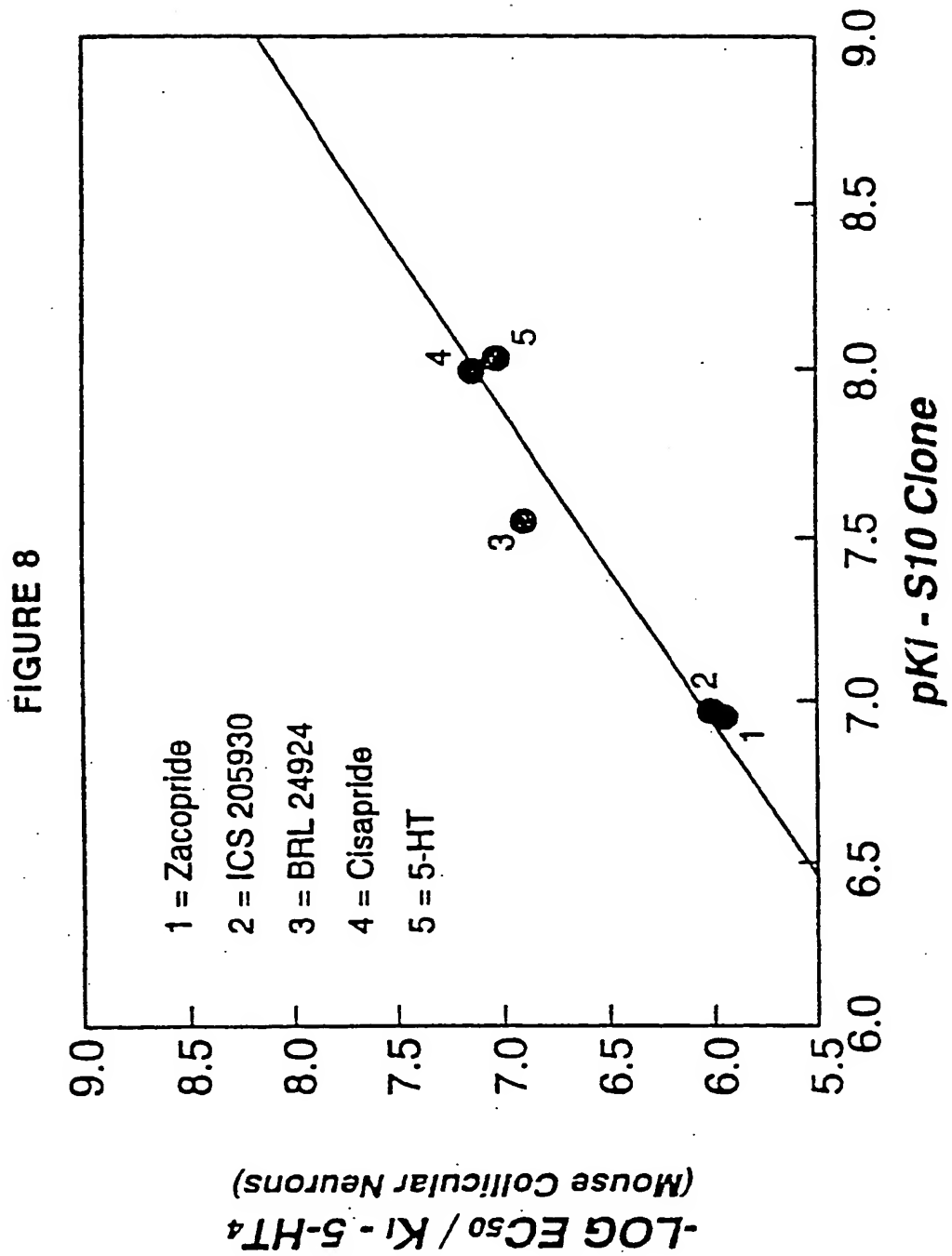

FIGURE 8

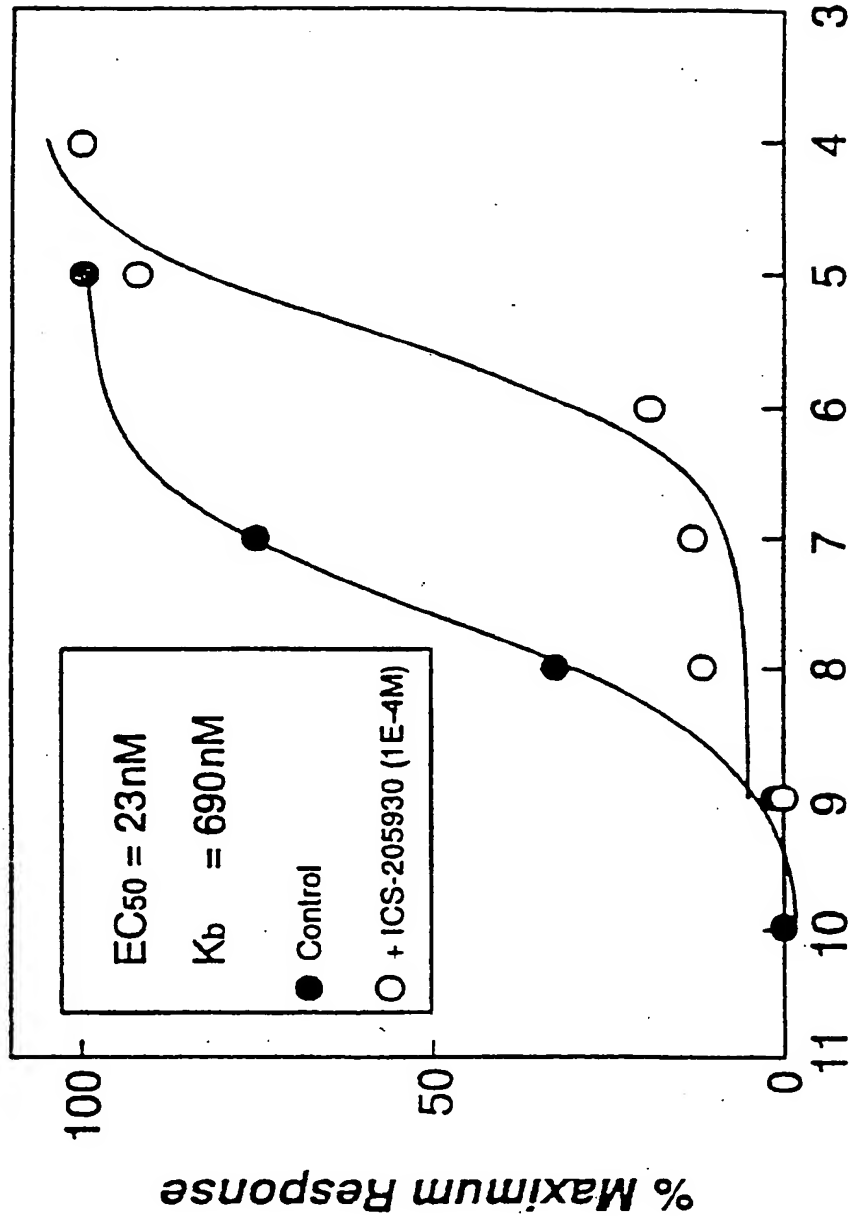

FIGURE 9

FIGURE 10

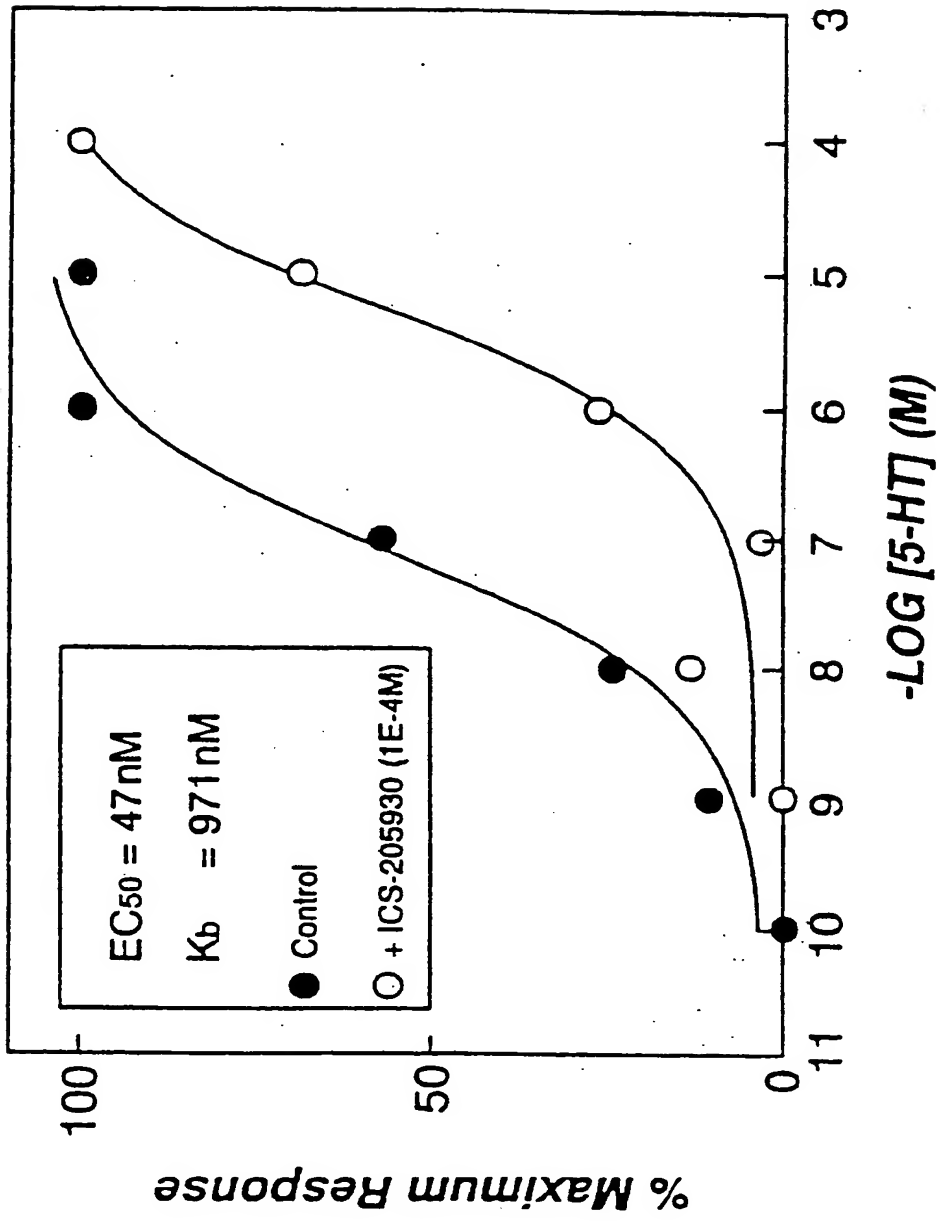
% Maximum Response
100
50
0
-LOG [5-HT] (M)
3
4
5
6
7
8
9
10
11
EC50 = 47nM
Kb = 971nM
Control
+ ICS-205930 (1E-4M)

FIGURE 11A

```
   1  TTGGTCTATAGGAACAAGATGACCCCTCTGCGCATCGCATTAATGCTGGGAGGCTGCTGG   60
  61  GTCATCCCCACGTTTATTTCTTTTCTCCCTATAATGCAAGGCTGGAATAACATTGGCATA  120
 121  ATTGATTTGATAGAAAAGAGGAAGTTCAACCAGAACTCTAACTCTACGTACTGTGTCTTC  180
 181  ATGGTCAACAAGCCCTACGCCATCACCTGCTCTGTGGTGGCCTTCTACATCCCATTTCTC  240
 241  CTCATGGTGCTGGCCTATTACCGCATCTATGTCACAGCTAAGGAGCATGCCCATCAGATC  300
 301  CAGATGTTACAACGGGCAGGAGCCTCCTCCGAGAGCAGGCCTCAGTCGGCAGACCAGCAT  360
 361  AGCACTCATCGCATGAGGACAGAGACCAAAGCAGCCAAGACCCTGTGCATCATCATGGGT  420
 421  TGCTTCTGCCTCTGCTGGGCACCATTCTTTGTCACCAATATTGTGGATCCTTTCATAGAC  480
 481  TACACTGTCCCTGGGCAGGTGTGGACTGCTTTCCTCTGGCTCGGCTATATCAATTCCGGG  540
 541  TTGAACCCTTTTCTCTACGCCTTCTTGAATAAGTCTTTTAGACGTGCCTTCCTCATCATC  600
 601  CTCTGCTGTGATGATGAGCGCTACCGAAGACCTTCCATTCTGGGCCAGACTGTCCCTTGT  660
 661  TCAACCACAACCATTAATGGATCCACACATGTACTAAGGTACACCGTTCTGCACAGGGGA  720
 721  CATCATCAGGAACTCGAGAAACTGCCCATACACAATGACCCAGAATCCCTGGAATCATGC  780
 781  TTCTGATTGAGG  792
```

FIGURE 11B

```
   1  L  V  Y  R  N  K  M  T  P  L  R  I  A  L  M  L  G  G  C  W   20
  21  V  I  P  T  F  I  S  F  L  P  I  M  Q  G  W  N  N  I  G  I   40
  41  I  D  L  I  E  K  R  K  F  N  Q  N  S  N  S  T  Y  C  V  F   60
  61  M  V  N  K  P  Y  A  I  T  C  S  V  V  A  F  Y  I  P  F  L   80
  81  L  M  V  L  A  Y  Y  R  I  Y  V  T  A  K  E  H  A  H  Q  I  100
 101  Q  M  L  Q  R  A  G  A  S  S  E  S  R  P  Q  S  A  D  Q  H  120
 121  S  T  H  R  M  R  T  E  T  K  A  A  K  T  L  C  I  I  M  G  140
 141  C  F  C  L  C  W  A  P  F  F  V  T  N  I  V  D  P  F  I  D  160
 161  Y  T  V  P  G  Q  V  W  T  A  F  L  W  L  G  Y  I  N  S  G  180
 181  L  N  P  F  L  Y  A  F  L  N  K  S  F  R  R  A  F  L  I  I  200
 201  L  C  C  D  D  E  R  Y  R  R  P  S  I  L  G  Q  T  V  P  C  220
 221  S  T  T  T  I  N  G  S  T  H  V  L  R  Y  T  V  L  H  R  G  240
 241  H  H  Q  E  L  E  K  L  P  I  H  N  D  P  E  S  L  E  S  C  260
 261  F  *                 262
```

FIGURE 12-1, 16/32
FIGURE 12-2, 17/32
FIGURE 12-3, 18/32

FIGURE 12-1

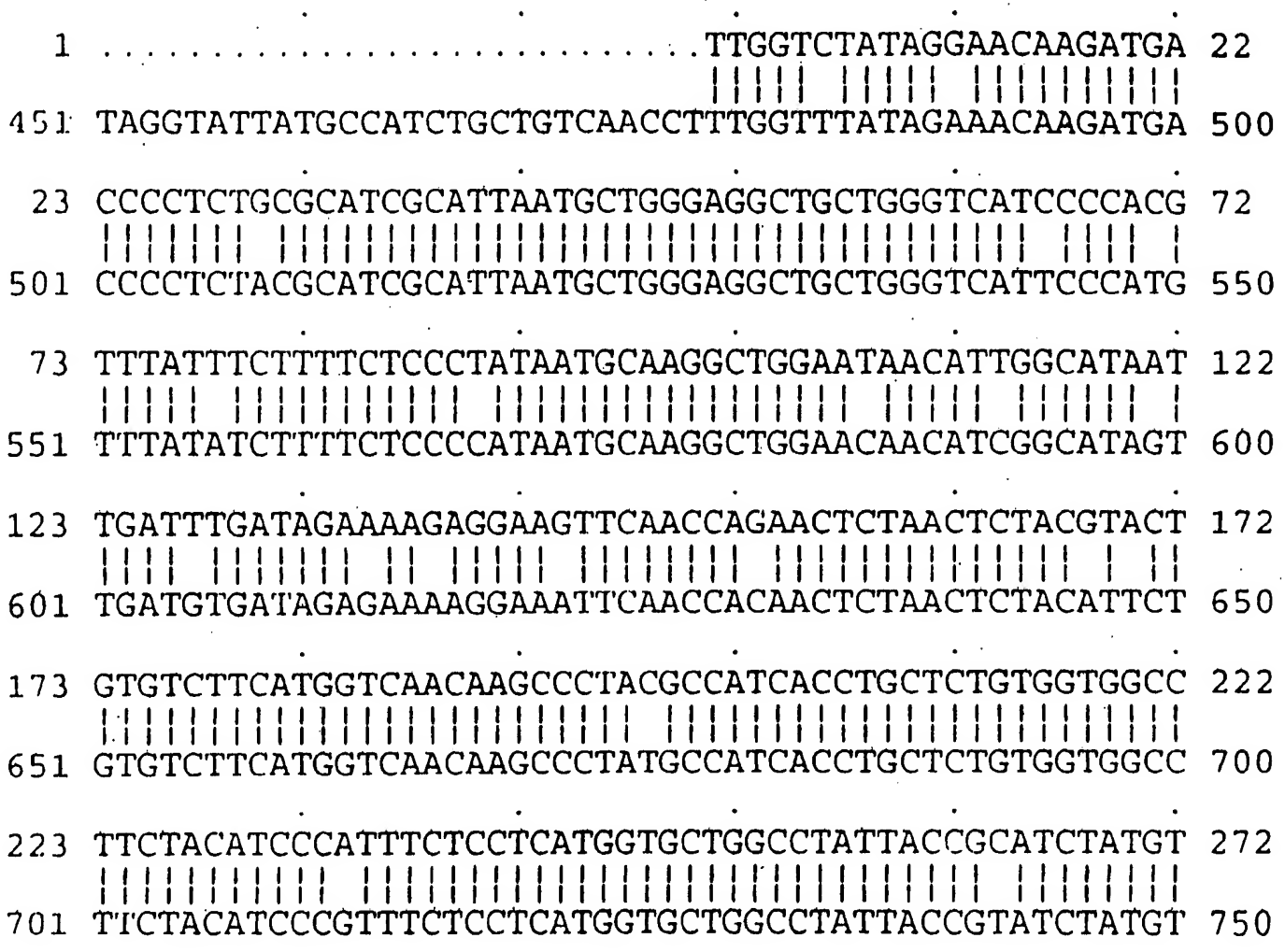

FIGURE 12-2

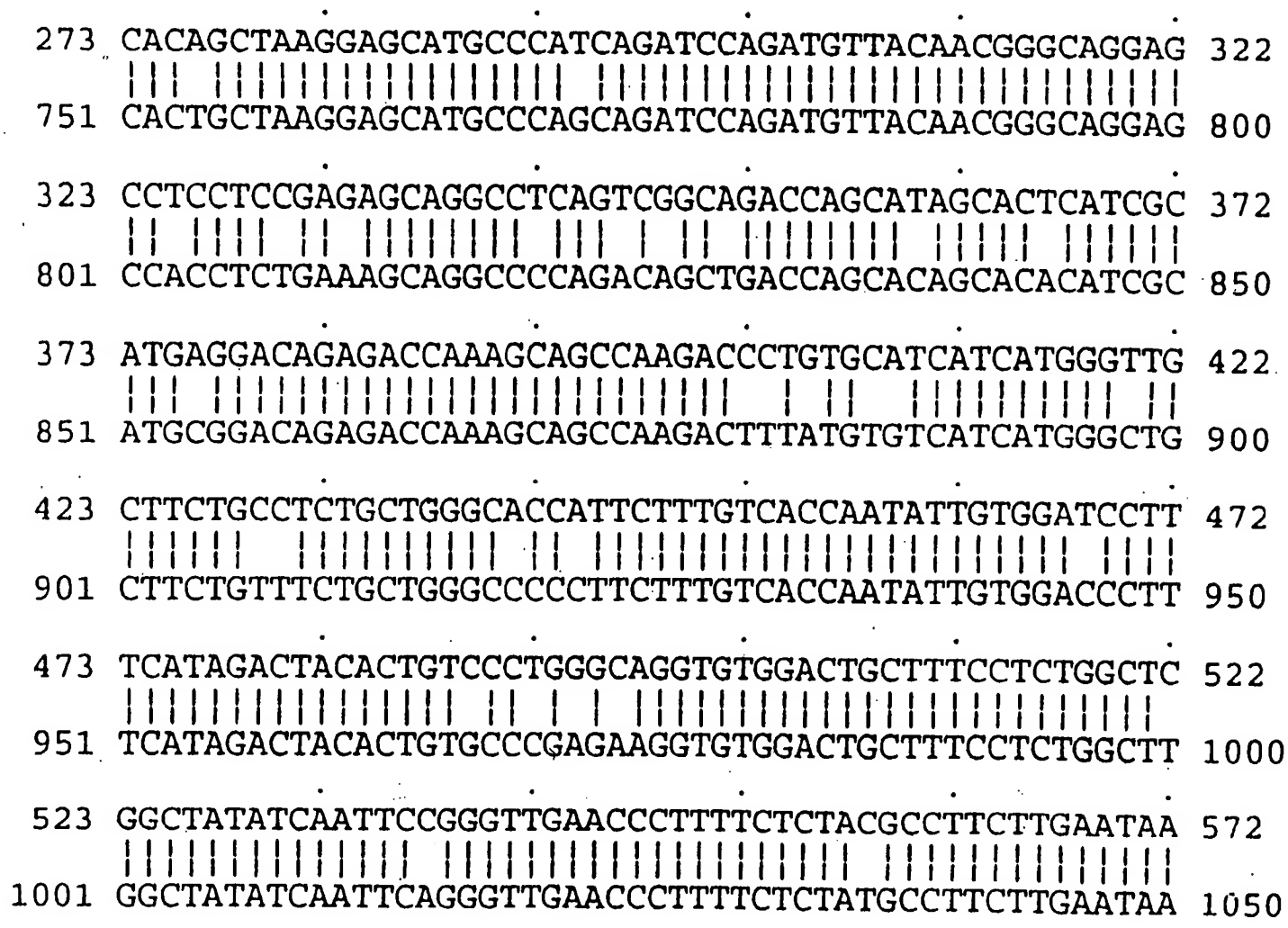

FIGURE 12-3

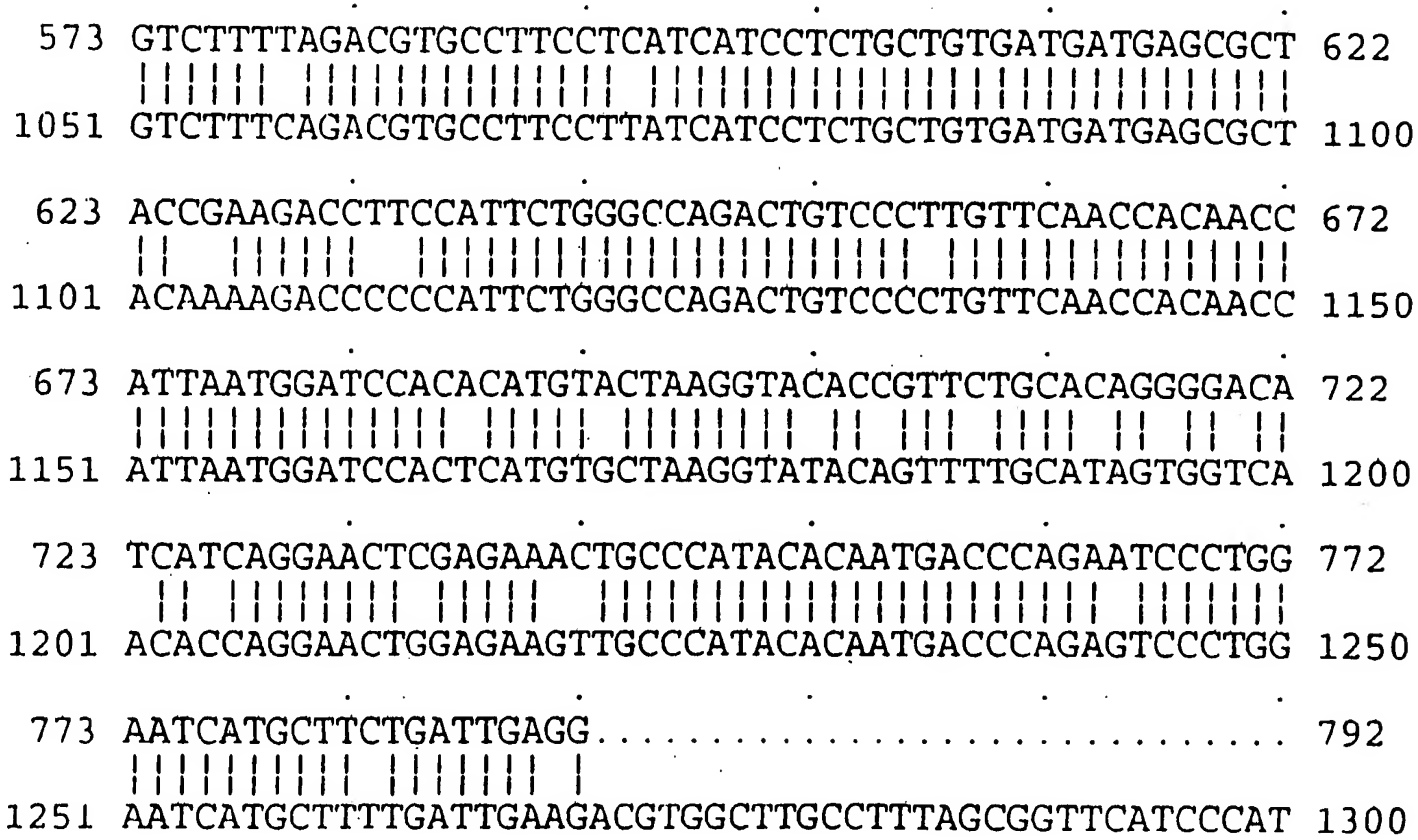

FIGURE 13

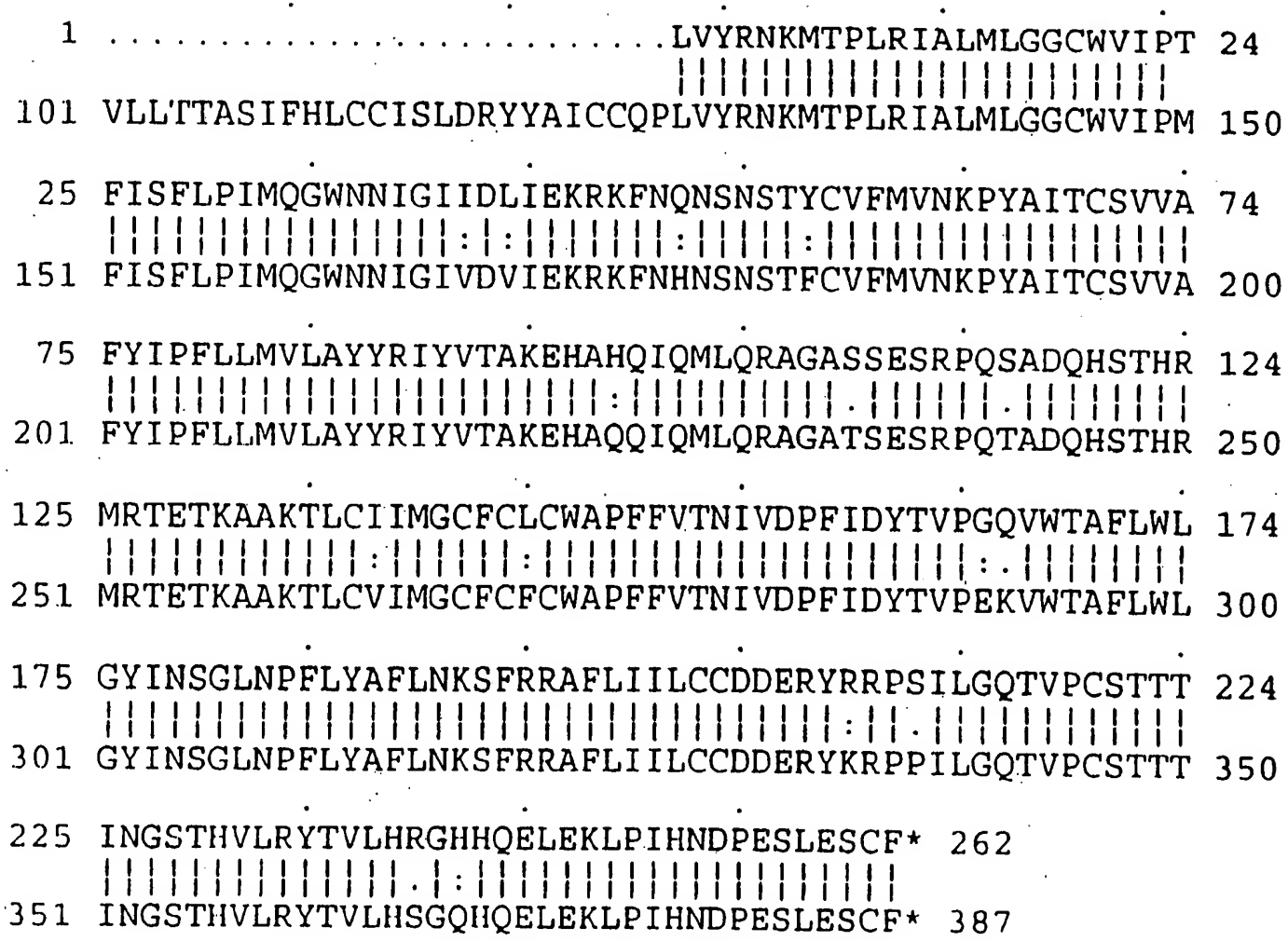

FIGURE 14A

FIGURE 14A, 20/32
FIGURE 14B, 21/32

```
   1  CCTGTAATGGACAAACTTGATGCTAATGTGAGTTCTGAGGAGGGTTTCGGGTCAGTGGAG   60
  61  AAGGTGGTGCTGCTCACGTTTCTCTCGACGGTTATCCTGATGGCCATCTTGGGGAACCTG  120
 121  CTGGTGATGGTGGCTGTGTGCTGGGACAGGCAGCTCAGGAAAATAAAAACAAATTATTTC  180
 181  ATTGTATCTCTTGCTTTTGCGGATCTGCTGGTTTCGGTGCTGGTGATGCCCTTTGGTGCC  240
 241  ATTGAGCTGGTTCAAGACATCTGGATTTATGGGGAGGTGTTTTGTCTTGTTCGGACATCT  300
 301  CTGGACGTCCTGCTCACAACGGCATCGATTTTTCACCTGTGCTGCATTTCTCTGGATAGG  360
 361  TATTACGCCATCTGCTGCCAGCCTTTGGTCTATAGGAACAAGATGACCCCTCTGCGCATC  420
 421  GCATTAATGCTGGGAGGCTGCTGGGTCATCCCCACGTTTATTTCTTTTCTCCCTATAATG  480
 481  CAAGGCTGGAATAACATTGGCATAATTGATTTGATAGAAAAGAGGAAGTTCAACCAGAAC  540
 541  TCTAACTCTACGTACTGTGTCTTCATGGTCAACAAGCCCTACGCCATCACCTGCTCTGTG  600
 601  GTGGCCTTCTACATCCCATTTCTCCTCATGGTGCTGGCCTATTACCGCATCTATGTCACA  660
 661  GCTAAGGAGCATGCCCATCAGATCCAGATGTTACAACGGGCAGGAGCCTCCTCCGAGAGC  720
 721  AGGCCTCAGTCGGCAGACCAGCATAGCACTCATCGCATGAGGACAGAGACCAAAGCAGCC  780
 781  AAGACCCTGTGCATCATCATGGGTTGCTTCTGCCTCTGCTGGGCACCATTCTTTGTCACC  840
 841  AATATTGTGGATCCTTTCATAGACTACACTGTCCCTGGGCAGGTGTGGACTGCTTTCCTC  900
 901  TGGCTCGGCTATATCAATTCCGGGTTGAACCCTTTTCTCTACGCCTTCTTGAATAAGTCT  960
 961  TTTAGACGTGCCTTCCTCATCATCCTCTGCTGTGATGATGAGCGCTACCGAAGACCTTCC 1020
1021  ATTCTGGGCCAGACTGTCCCTTGTTCAACCACAACCATTAATGGATCCACACATGTACTA 1080
1081  AGGGATGCAGTGGAGTGTGGTGGCCAGTGGGAGAGTCAGTGTCACCCGCCAGCAACTTCT 1140
1141  CCTTTGGTGGCTGCTCAGCCCAGTGACACTTAGGCCCCTGGGACAATGACCCAGAAGACA 1200
1201  GCCATGCCTCCGAAAGAGGGCCAGGTCCTAAGCTGCTGCTTGTGCGCGACTGCACCCGGC 1260
1261  ATTCTCTTCACCTGAGGCTTTCCGTCCGCCAGTGCAGGAACCCGGTGCTCGCTGGG  1316
```

FIGURE 14B

1			M	D	K	L	D	A	N	V	S	S	E	E	G	F	G	S	V	E	20
21	K	V	V	L	L	T	F	L	S	T	V	I	L	M	A	I	L	G	N	L	40
41	L	V	M	V	A	V	C	W	D	R	Q	L	R	K	I	K	T	N	Y	F	60
61	I	V	S	L	A	F	A	D	L	L	V	S	V	L	V	M	P	F	G	A	80
81	I	E	L	V	Q	D	I	W	I	Y	G	E	V	F	C	L	V	R	T	S	100
101	L	D	V	L	L	T	T	A	S	I	F	H	L	C	C	I	S	L	D	R	120
121	Y	Y	A	I	C	C	Q	P	L	V	Y	R	N	K	M	T	P	L	R	I	140
141	A	L	M	L	G	G	C	W	V	I	P	T	F	I	S	F	L	P	I	M	160
161	Q	G	W	N	N	I	G	I	I	D	L	I	E	K	R	K	F	N	Q	N	180
181	S	N	S	T	Y	C	V	F	M	V	N	K	P	Y	A	I	T	C	S	V	200
201	V	A	F	Y	I	P	F	L	L	M	V	L	A	Y	Y	R	I	Y	V	T	220
221	A	K	E	H	A	H	Q	I	Q	M	L	Q	R	A	G	A	S	S	E	S	240
241	R	P	Q	S	A	D	Q	H	S	T	H	R	M	R	T	E	T	K	A	A	260
261	K	T	L	C	I	I	M	G	C	F	C	L	C	W	A	P	F	F	V	T	280
281	N	I	V	D	P	F	I	D	Y	T	V	P	G	Q	V	W	T	A	F	L	300
301	W	L	G	Y	I	N	S	G	L	N	P	F	L	Y	A	F	L	N	K	S	320
321	F	R	R	A	F	L	I	I	L	C	C	D	D	E	R	Y	R	R	P	S	340
341	I	L	G	Q	T	V	P	C	S	T	T	T	I	N	G	S	T	H	V	L	360
361	R	D	A	V	E	C	G	G	Q	W	E	S	Q	C	H	P	P	A	T	S	380
381	P	L	V	A	A	Q	P	S	D	T	*										400

FIGURE 15-1

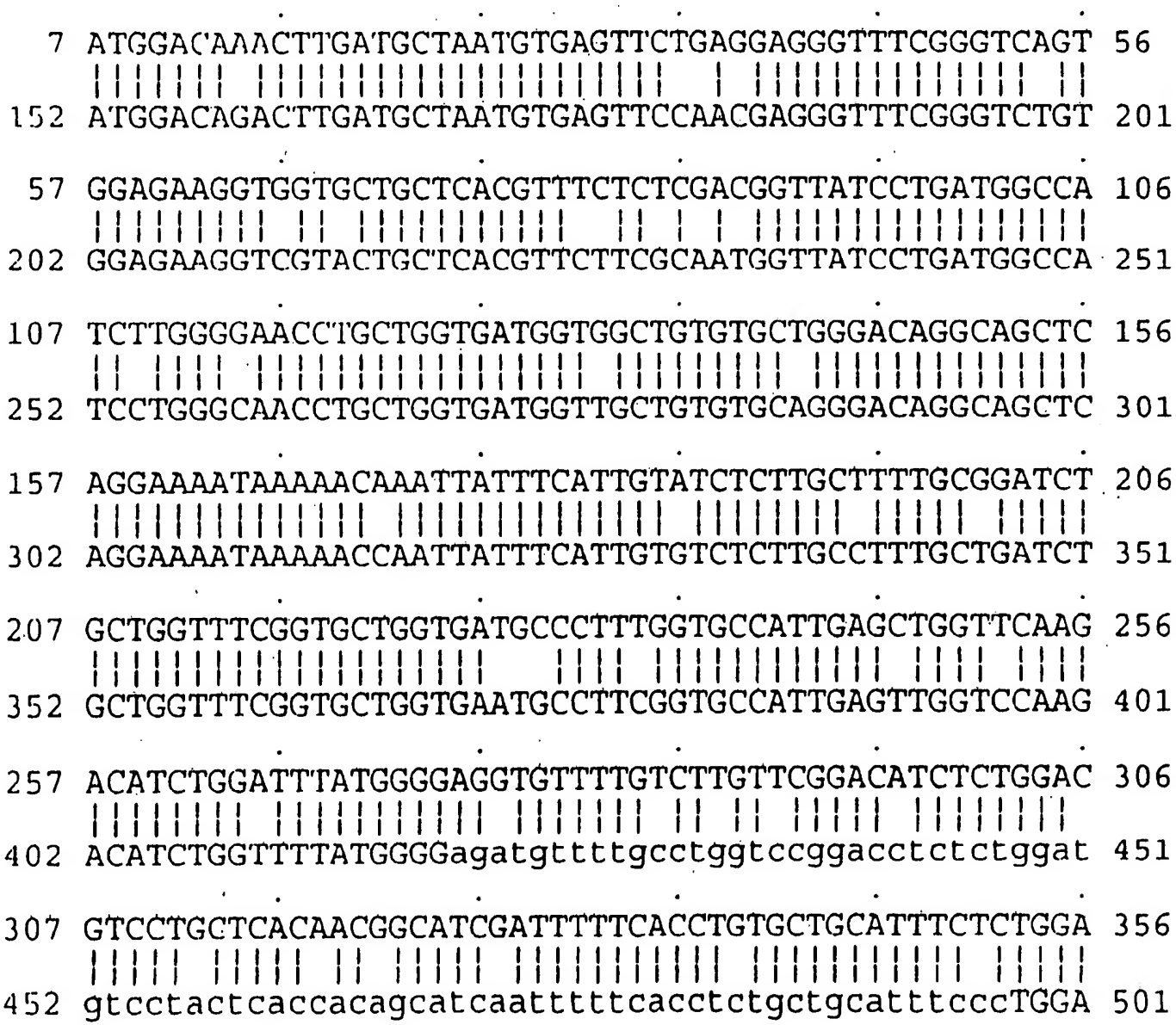

FIGURE 15-2

```
357 TAGGTATTACGCCATCTGCTGCCAGCCTTTGGTCTATAGGAACAAGATGA 406
    ||||||||| |||||||||| || |||||||| ||||| ||||||||||
502 TAGGTATTATGCCATCTGCTGTCAACCTTTGGTTTATAGAAACAAGATGA 551

407 CCCCTCTGCGCATCGCATTAATGCTGGGAGGCTGCTGGGTCATCCCCACG 456
    ||||||| ||||||||||||||||||||||||||||||| |||| |
552 CCCCTCTACGCATCGCATTAATGCTGGGAGGCTGCTGGGTCATTCCCATG 601

457 TTTATTTCTTTTCTCCCTATAATGCAAGGCTGGAATAACATTGGCATAAT 506
    ||||| |||||||||| |||||||||||||||| ||||| |||||| |
602 TTTATATCTTTTCTCCCCATAATGCAAGGCTGGAACAACATCGGCATAGT 651

507 TGATTTGATAGAAAAGAGGAAGTTCAACCAGAACTCTAACTCTACGTACT 556
    |||| ||||||| || ||||| |||||||| |||||||||||||| | ||
652 TGATGTGATAGAGAAAAGGAAATTCAACCACAACTCTAACTCTACATTCT 701

557 GTGTCTTCATGGTCAACAAGCCCTACGCCATCACCTGCTCTGTGGTGGCC 606
    |||||||||||||||||||||||| ||||||||||||||||||||||||
702 GTGTCTTCATGGTCAACAAGCCCTATGCCATCACCTGCTCTGTGGTGGCC 751

607 TTCTACATCCCATTTCTCCTCATGGTGCTGGCCTATTACCGCATCTATGT 656
    |||||||||||| |||||||||||||||||||||||||||| |||||||||
752 TTCTACATCCCGTTTCTCCTCATGGTGCTGGCCTATTACCGTATCTATGT 801
```

FIGURE 15-3

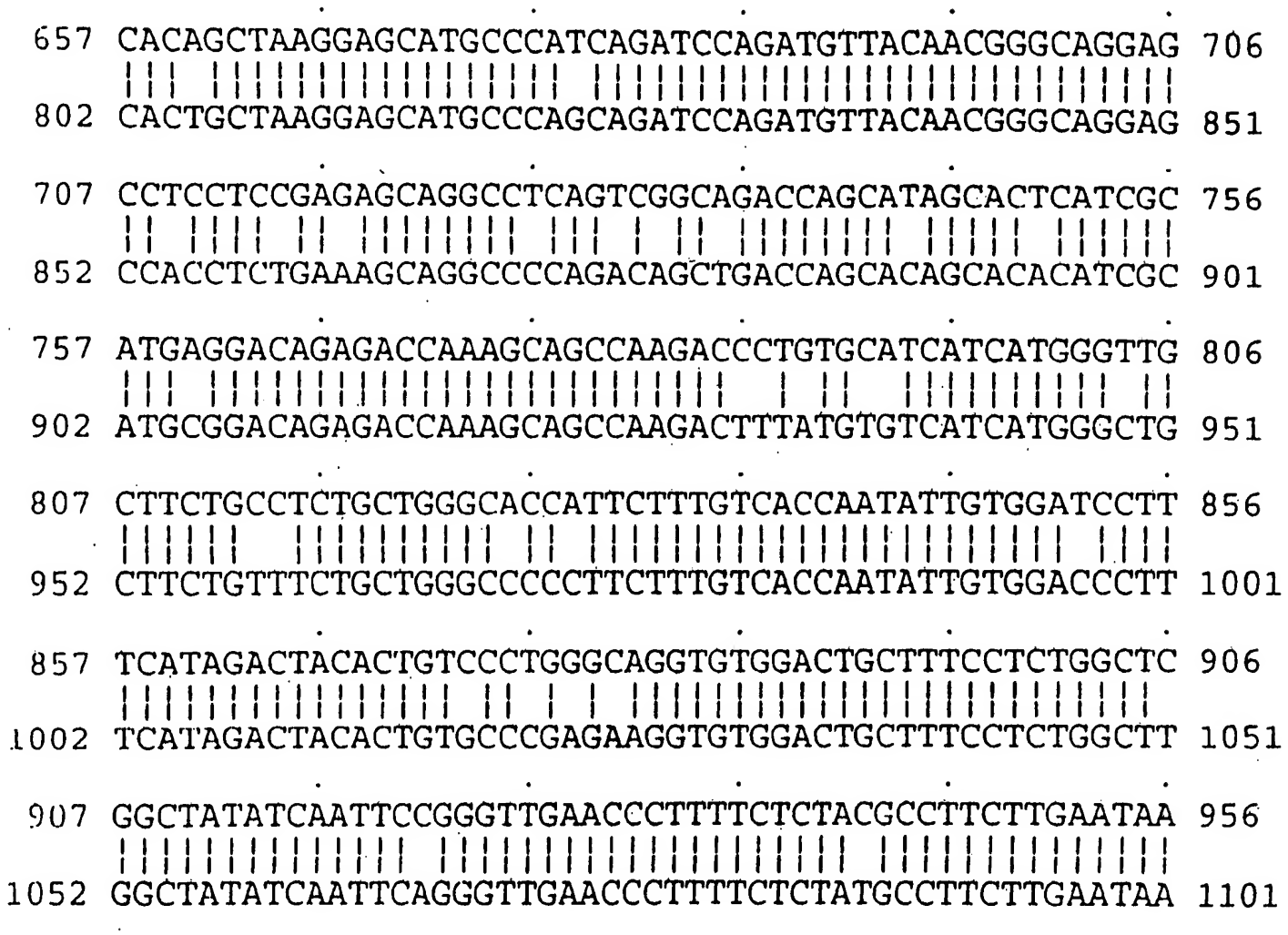
657 CACAGCTAAGGAGCATGCCCATCAGATCCAGATGTTACAACGGGCAGGAG 706
802 CACTGCTAAGGAGCATGCCCAGCAGATCCAGATGTTACAACGGGCAGGAG 851
707 CCTCCTCCGAGAGCAGGCCTCAGTCGGCAGACCAGCATAGCACTCATCGC 756
852 CCACCTCTGAAAGCAGGCCCCAGACAGCTGACCAGCACAGCACACATCGC 901
757 ATGAGGACAGAGACCAAAGCAGCCAAGACCCTGTGCATCATCATGGGTTG 806
902 ATGCGGACAGAGACCAAAGCAGCCAAGACTTTATGTGTCATCATGGGCTG 951
807 CTTCTGCCTCTGCTGGGCACCATTCTTTGTCACCAATATTGTGGATCCTT 856
952 CTTCTGTTTCTGCTGGGCCCCCTTCTTTGTCACCAATATTGTGGACCCTT 1001
857 TCATAGACTACACTGTCCCTGGGCAGGTGTGGACTGCTTTCCTCTGGCTC 906
1002 TCATAGACTACACTGTGCCCGAGAAGGTGTGGACTGCTTTCCTCTGGCTT 1051
907 GGCTATATCAATTCCGGGTTGAACCCTTTTCTCTACGCCTTCTTGAATAA 956
1052 GGCTATATCAATTCAGGGTTGAACCCTTTTCTCTATGCCTTCTTGAATAA 1101

FIGURE 15-4

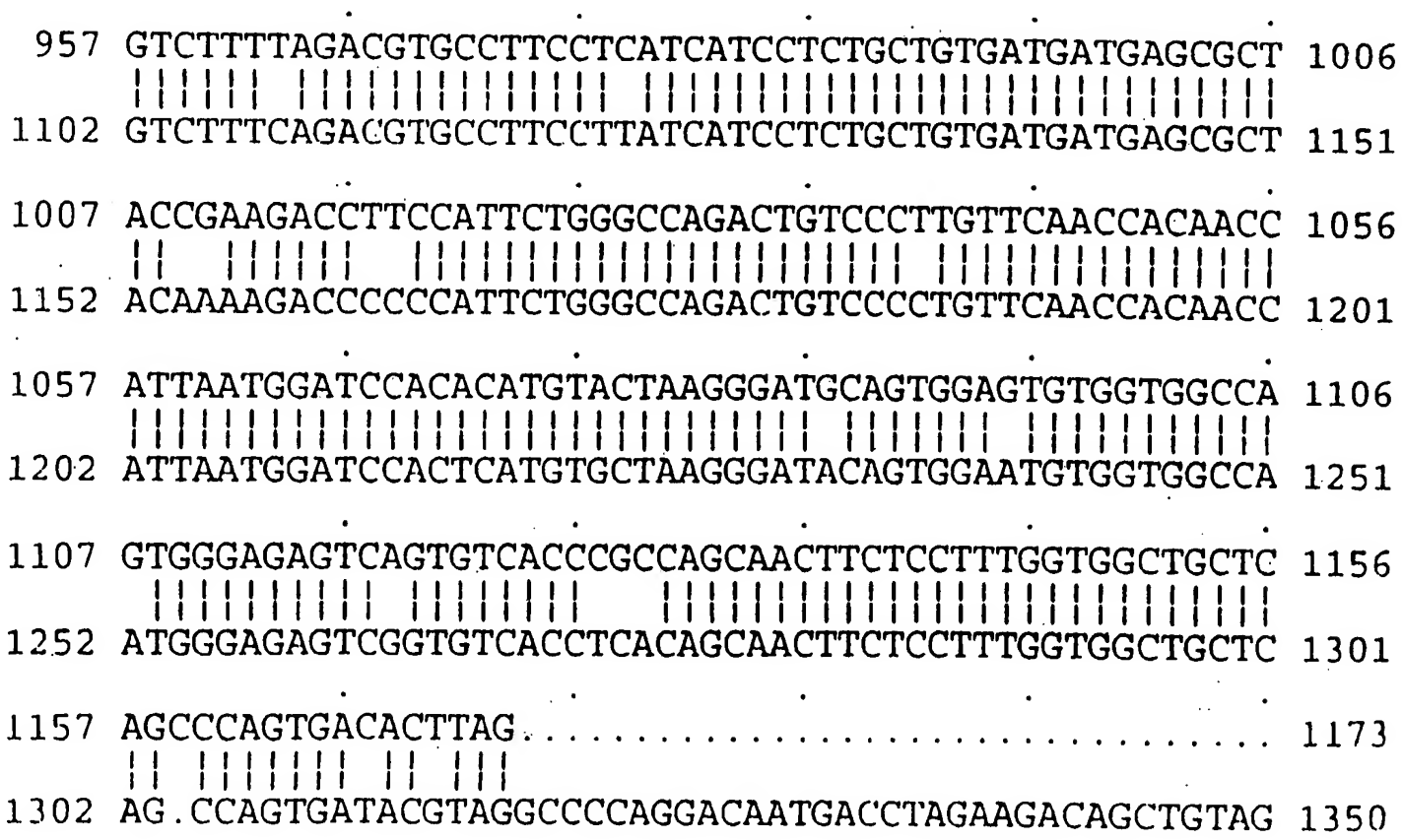

FIGURE 16-1

FIGURE 16-1, 26/32
FIGURE 16-2, 27/32

```
  1 MDKLDANVSSEEGFGSVEKVVLLTFLSTVILMAILGNLLVMVAVCWDRQL 50
    ||:|||||||:||||||||||||||:. ||||||||||||||||||:||||
  1 MDRLDANVSSNEGFGSVEKVVLLTFFAMVILMAILGNLLVMVAVCRDRQL 50

 51 RKIKTNYFIVSLAFADLLVSVLVMPFGAIELVQDIWIYGEVFCLVRTSLD 100
    |||||||||||||||||||||||| :||||||||||:|||:|||||||||
 51 RKIKTNYFIVSLAFADLLVSVLVNAFGAIELVQDIWFYGEMFCLVRTSLD 100

101 VLLTTASIFHLCCISLDRYYAICCQPLVYRNKMTPLRIALMLGGCWVIPT 150
    ||||||||||||||||||||||||||||||||||||||||||||||||||
101 VLLTTASIFHLCCISLDRYYAICCQPLVYRNKMTPLRIALMLGGCWVIPM 150

151 FISFLPIMQGWNNIGIIDLIEKRKFNQNSNSTYCVFMVNKPYAITCSVVA 200
    ||||||||||||||||:|:|||||||:|||||:||||||||||||||||
151 FISFLPIMQGWNNIGIVDVIEKRKFNHNSNSTFCVFMVNKPYAITCSVVA 200

201 FYIPFLLMVLAYYRIYVTAKEHAHQIQMLQRAGASSESRPQSADQHSTHR 250
    ||||||||||||||||||||:||||||||||.||||||.||||||||||
201 FYIPFLLMVLAYYRIYVTAKEHAQQIQMLQRAGATSESRPQTADQHSTHR 250
```

FIGURE 16-2

```
251 MRTETKAAKTLCIIMGCFCLCWAPFFVTNIVDPFIDYTVPGQVWTAFLWL 300
    ||||||||||||:||||||:||||||||||||||||||||:.||||||||
251 MRTETKAAKTLCVIMGCFCFCWAPFFVTNIVDPFIDYTVPEKVWTAFLWL 300

301 GYINSGLNPFLYAFLNKSFRRAFLIILCCDDERYRRPSILGQTVPCSTTT 350
    ||||||||||||||||||||||||||||||||||:||.||||||||||||
301 GYINSGLNPFLYAFLNKSFRRAFLIILCCDDERYKRPPILGQTVPCSTTT 350

351 INGSTHVLRDAVECGGQWESQCHPPATSPLVAAQPSDT*..........  389
    ||||||||||.||||||||.|| .||||||||||
351 INGSTHVLRDTVECGGQWESRCHLTATSPLVAAQPVIRRPQDNDLEDSCS 400

401 LKRSQS*.....................................  406
```

FIGURE 17-1


```
357 TAGGTATTACGCCATCTGCTGCCAGCCTTTGGTCTATAGGAACAAGATGA 406
                                  ||||||||||||||||||||||||
  1 .............................TTGGTCTATAGGAACAAGATGA  22

407 CCCCTCTGCGCATCGCATTAATGCTGGGAGGCTGCTGGGTCATCCCCACG 456
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
 23 CCCCTCTGCGCATCGCATTAATGCTGGGAGGCTGCTGGGTCATCCCCACG  72

457 TTTATTTCTTTTCTCCCTATAATGCAAGGCTGGAATAACATTGGCATAAT 506
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
 73 TTTATTTCTTTTCTCCCTATAATGCAAGGCTGGAATAACATTGGCATAAT 122

507 TGATTTGATAGAAAAGAGGAAGTTCAACCAGAACTCTAACTCTACGTACT 556
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
123 TGATTTGATAGAAAAGAGGAAGTTCAACCAGAACTCTAACTCTACGTACT 172

557 GTGTCTTCATGGTCAACAAGCCCTACGCCATCACCTGCTCTGTGGTGGCC 606
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
173 GTGTCTTCATGGTCAACAAGCCCTACGCCATCACCTGCTCTGTGGTGGCC 222

607 TTCTACATCCCATTTCTCCTCATGGTGCTGGCCTATTACCGCATCTATGT 656
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
223 TTCTACATCCCATTTCTCCTCATGGTGCTGGCCTATTACCGCATCTATGT 272

657 CACAGCTAAGGAGCATGCCCATCAGATCCAGATGTTACAACGGGCAGGAG 706
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
273 CACAGCTAAGGAGCATGCCCATCAGATCCAGATGTTACAACGGGCAGGAG 322
```

FIGURE 17-2

```
707 CCTCCTCCGAGAGCAGGCCTCAGTCGGCAGACCAGCATAGCACTCATCGC 756
    ||||||||||||||||||||||||||||||||||||||||||||||||||
323 CCTCCTCCGAGAGCAGGCCTCAGTCGGCAGACCAGCATAGCACTCATCGC 372

757 ATGAGGACAGAGACCAAAGCAGCCAAGACCCTGTGCATCATCATGGGTTG 806
    ||||||||||||||||||||||||||||||||||||||||||||||||||
373 ATGAGGACAGAGACCAAAGCAGCCAAGACCCTGTGCATCATCATGGGTTG 422

807 CTTCTGCCTCTGCTGGGCACCATTCTTTGTCACCAATATTGTGGATCCTT 856
    ||||||||||||||||||||||||||||||||||||||||||||||||||
423 CTTCTGCCTCTGCTGGGCACCATTCTTTGTCACCAATATTGTGGATCCTT 472

857 TCATAGACTACACTGTCCCTGGGCAGGTGTGGACTGCTTTCCTCTGGCTC 906
    ||||||||||||||||||||||||||||||||||||||||||||||||||
473 TCATAGACTACACTGTCCCTGGGCAGGTGTGGACTGCTTTCCTCTGGCTC 522

907 GGCTATATCAATTCCGGGTTGAACCCTTTTCTCTACGCCTTCTTGAATAA 956
    ||||||||||||||||||||||||||||||||||||||||||||||||||
523 GGCTATATCAATTCCGGGTTGAACCCTTTTCTCTACGCCTTCTTGAATAA 572

957 GTCTTTTAGACGTGCCTTCCTCATCATCCTCTGCTGTGATGATGAGCGCT 1006
    ||||||||||||||||||||||||||||||||||||||||||||||||||
573 GTCTTTTAGACGTGCCTTCCTCATCATCCTCTGCTGTGATGATGAGCGCT 622
```

FIGURE 17-3

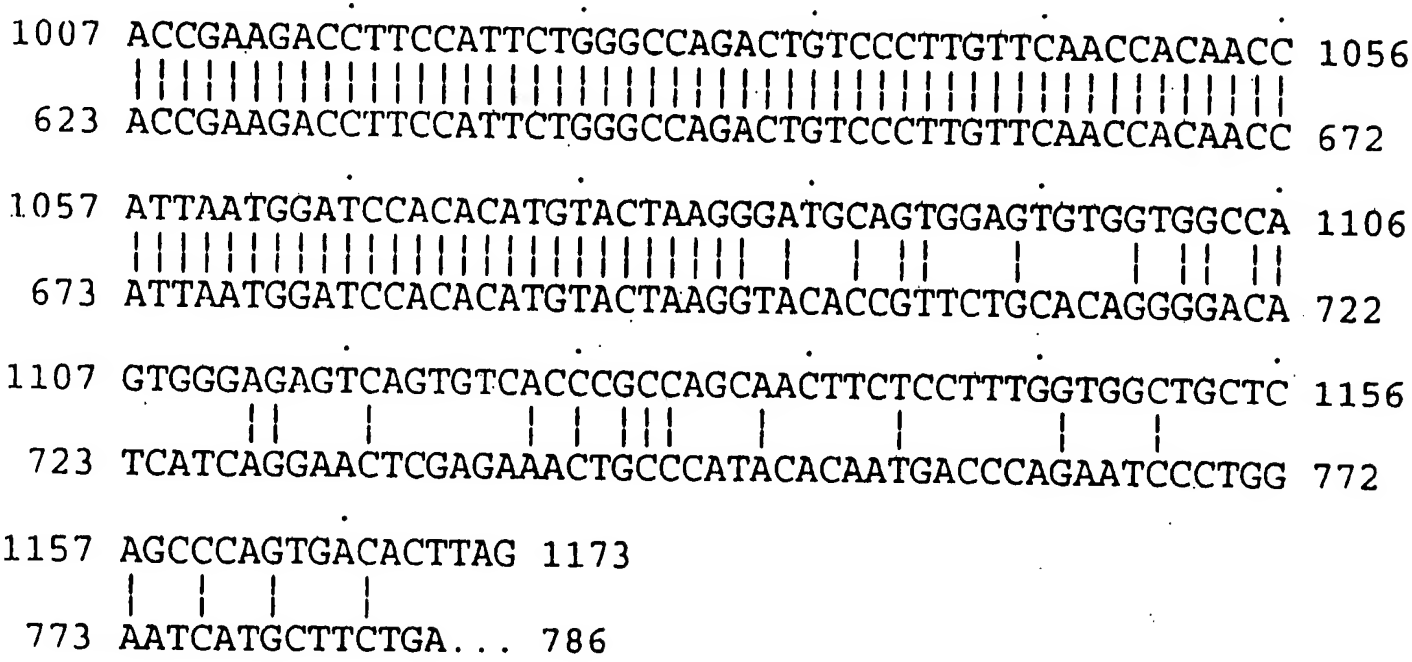

FIGURE 18

```
101 VLLTTASIFHLCCISLDRYYAICCQPLVYRNKMTPLRIALMLGGCWVIPT 150
                            ||||||||||||||||||||||||
  1 ........................LVYRNKMTPLRIALMLGGCWVIPT  24

151 FISFLPIMQGWNNIGIIDLIEKRKFNQNSNSTYCVFMVNKPYAITCSVVA 200
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
 25 FISFLPIMQGWNNIGIIDLIEKRKFNQNSNSTYCVFMVNKPYAITCSVVA  74

201 FYIPFLLMVLAYYRIYVTAKEHAHQIQMLQRAGASSESRPQSADQHSTHR 250
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
 75 FYIPFLLMVLAYYRIYVTAKEHAHQIQMLQRAGASSESRPQSADQHSTHR 124

251 MRTETKAAKTLCIIMGCFCLCWAPFFVTNIVDPFIDYTVPGQVWTAFLWL 300
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
125 MRTETKAAKTLCIIMGCFCLCWAPFFVTNIVDPFIDYTVPGQVWTAFLWL 174

301 GYINSGLNPFLYAFLNKSFRRAFLIILCCDDERYRRPSILGQTVPCSTTT 350
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
175 GYINSGLNPFLYAFLNKSFRRAFLIILCCDDERYRRPSILGQTVPCSTTT 224

351 INGSTHVLRDAVECGGQWESQCHPPATSPLVAAQPSDT* 389
    |||||||||  .|    |: :.  . |  ..  . :.:
225 INGSTHVLRYTVLHRGHHQELEKLPIHNDPESLESCF*. 262
```

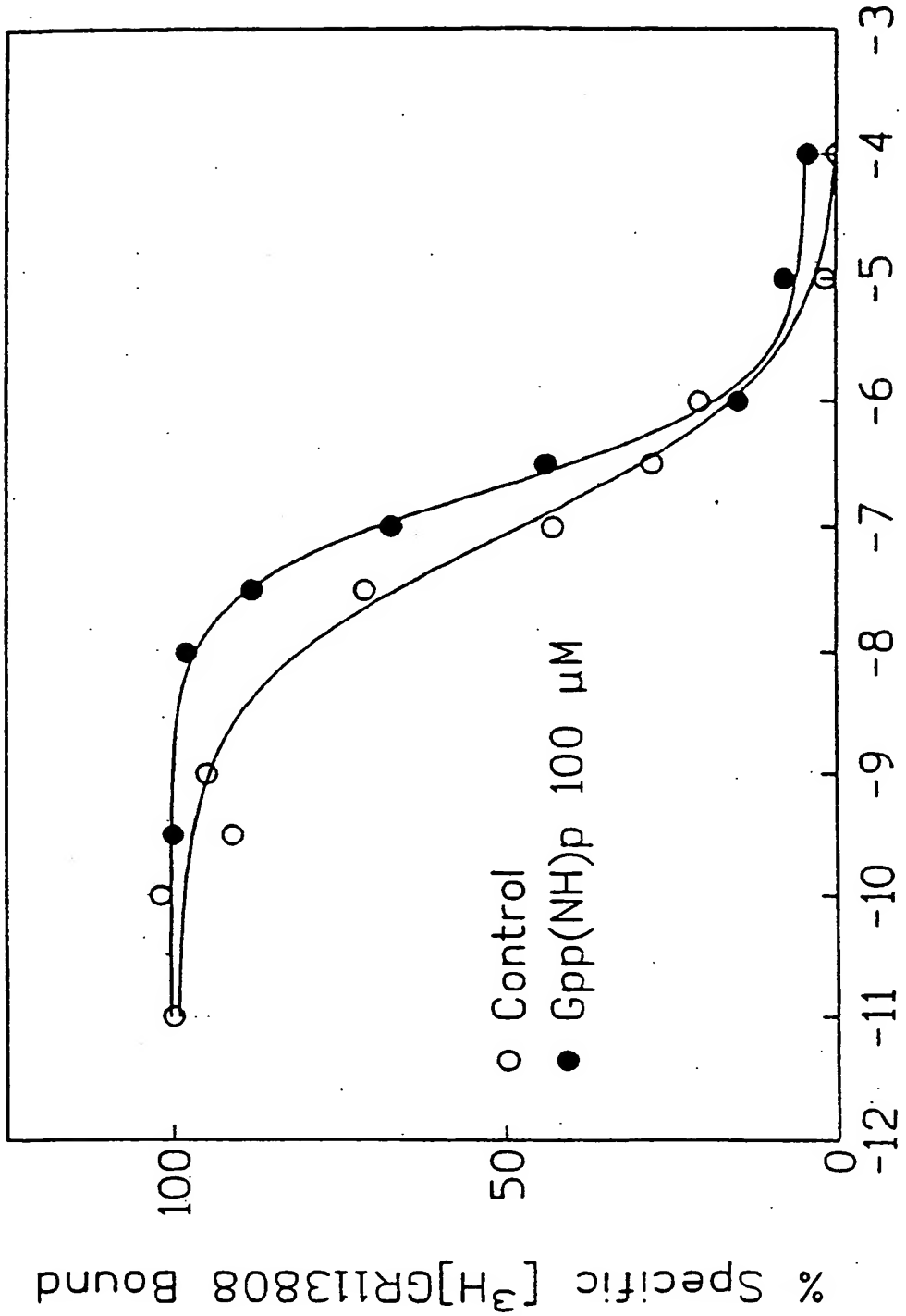

FIGURE 19

INTERNATIONAL SEARCH REPORT

International application No.
T/US93/12586

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 1/15, 1/21, 5/10, 15/00, 15/12
US CL : 435/6, 172.3, 240.2, 252.3, 255.1, 320.1; 536/23.5, 24.3, 24.31
According to International Patent Classification (IPC) or to both national classificati n and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system foll wed by classification symbols)

U.S. : 435/6, 172.3, 240.2, 252.3, 255.1, 320.1; 536/23.5, 24.3, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (files 5, 155, 351, 357, 358) search terms: serotonin, receptor, DNA, 5-HT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,985,352 (JULIUS ET AL) 15 January 1991, see Figure 1.	25-28
A, P	US, A, 5,242,822 (MARULLO ET AL) 07 September 1993, see abstract and claims.	1-37, 40-42
A	FEBS, Volume 312, Number 2,3, issued November 1992, Loric et al, "New mouse 5-HT2-like receptor", pages 203-207, see entire document.	1-37 and 40-42

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
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"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
07 June 1994

Date of mailing of the international search report
JUN 20 1994

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M. Kyza for
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/12586

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims N s.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-37 and 40-42

Remark n Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I.Claims 1-37 and 40-42, drawn to nucleic acid sequences for the 5-HT4 receptor, vectors, host cells, probes, antisense nucleic acids, and a method for detecting expression of a mammalian 5-HT4 receptor, classified in at least, for example, Class 536, subclass 23.5.

II.Claims 38-39, drawn to a method of isolating a gene, classified in at least, for example, Class 435, subclass 172.3.

III.Claims 43-44, drawn to a 5-HT4 receptor protein, classified in at least, for example, Class 530, subclass 350.

IV.Claims 45-48, drawn to a method of production of a mammalian 5-HT4 receptor, classified in at least, for example, Class 435, subclass 69.1.

V.Claims 49-54 and 61, drawn to an antibody to a 5-HT4 receptor protein, classified in at least, for example, Class 530, subclass 387.1.

VI.Claim 55, drawn to a pharmaceutical composition to alleviate abnormalities resulting from overexpression of a 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.

VII.Claim 56, drawn to a pharmaceutical composition to alleviate abnormalities resulting from underexpression of a 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.

VIII.Claims 57-60, drawn to pharmaceutical compositions containing oligonucleotides, classified in at least, for example, Class 514, subclass 44.

IX.Claims 62-66, drawn to transgenic animals, classified in at least, for example, Class 800, subclass 2.

X.Claims 67-68, drawn to a method for determining physiological effects of varying levels of 5-HT4 receptors using transgenic animals, classified in at least, for example, Class 514, subclass 44.

XI.Claims 69-70, drawn to a method for determining specific binding using whole cells, classified in at least, for example, Class 435, subclass 7.2.

XII.Claims 71-72, drawn to a method of screening compounds to identify drugs using whole cells, classified in at least, for example, Class 435, subclass 7.2.

XIII. Claims 73-74, drawn to a method for determining specific binding using cell extracts, classified in at least, forexample, Class 435, subclass 7.1.

XIV.Claims 75-76, drawn to a method of screening compounds to identify drugs using membrane fractions, classified in at least, for example, Class 435, subclass 7.1.

XV.Claims 77-82, drawn to a method for identifying a compound using whole cells and activation blockade, classified in at least, for example, Class 435, subclass 7.2.

XVI.Claims 83-84, drawn to compounds and pharmaceutical compositions, classified in at least, for example, Class 514, subclass 2.

XVII.Claim 85, drawn to a method for detecting 5-HT4 receptor on the cell surface using an antibody, classified in at least, for example, Class 435, subclass 7.2.

XVIII.Claim 86, drawn to a method for treating an abnormal condition related to excess activity of 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.

XIX.Claim 87, drawn to a method for treating an abnormal condition alleviated by increasing the activity of 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.

XX.Claims 88-89, drawn to a method for diagnosing a predisposition to a disorder associated with expression of 5-HT receptor alleles, classified in at least, for example, Class 435, subclass 6.

XXI. Claim 90, drawn to a method of identifying a substance that alleviates abnormalities from overexpression of 5-HT4 receptors using transgenic animals, classified in at least, for example, Class 424, subclass 9.

XXII. Claim 91, drawn to a method of identifying a substance that alleviates abnormalities from underexpression of 5-HT4 receptors using transgenic animals, classified in at least, for example, Class 424, subclass 9.

XXIII. Claim 92, drawn to a method of treating abnormalities alleviated by reduced expression of 5-HT4, classified in least, for example, Class 514, subclass 44.

XXIV. Claim 93, drawn to a method of treating abnormalities resulting from underexpression of 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.

PCT

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C12N 1/15, 1/21, 5/10, 15/00,15/12

A3

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(72) Inventors; and
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(74) Agent: COBERT, Robert, J.; Cooper & Dunham, 30 Rockefeller Plaza, New York, NY 10112 (US).

(81) Designated States: AU, CA, FI, HU, JP, KR, NO, NZ, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(88) Date of publication of the international search report:
18 August 1994 (18.08.94)

(54) Title: DNA ENCODING 5-HT$_4$ SEROTONIN RECEPTORS AND USES THEREOF

(57) Abstract

This invention provides an isolated nucleic acid molecule encoding a mammalian 5-HT$_4$ receptor and an isolated nucleic acid molecule encoding a human 5-HT$_4$ receptor, an isolated protein which is a mammalian 5-HT$_4$ receptor, an isolated protein which is a human 5-HT$_4$ receptor, vectors comprising an isolated nucleic acid molecule encoding a mammalian 5-HT$_4$ receptor, vectors comprising an isolated nucleic acid molecule encoding a human 5-HT$_4$ receptor, mammalian cells comprising such vectors, antibodies directed to the 5-HT$_4$ receptor, nucleic acid probes useful for detecting nucleic acid encoding a mammalian or human 5-HT$_4$ receptor, antisense oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a mammalian or human 5-HT$_4$ receptor, pharmaceutical compounds related to the human 5-HT$_4$ receptor, and nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian or human 5-HT$_4$ receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatments for alleviating abnormalities associated with a human 5-HT$_4$ receptor.

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BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 1/15, 1/21, 5/10, 15/[redacted]5/12

US Cl. : 435/6, 172.3, 240.2, 252.3, [redacted]1, 320.1; 536/23.5, 24.3, 24.31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 172.3, 240.2, 252.3, 255.1, 320.1; 536/23.5, 24.3, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (files 5, 155, 351, 357, 358) search terms: serotonin, receptor, DNA, 5-HT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,985,352 (JULIUS ET AL) 15 January 1991, see Figure 1.	25-28
A, P	US, A, 5,242,822 (MARULLO ET AL) 07 September 1993, see abstract and claims.	1-37, 40-42
A	FEBS, Volume 312, Number 2,3, issued November 1992, Loric et al, "New mouse 5-HT2-like receptor", pages 203-207, see entire document.	1-37 and 40-42

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 June 1994	JUN 20 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Marianne Porta Allen M. Kyza for Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-37 and 40-42

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

I.Claims 1-37 and 40-42, drawn to nucleic acid sequences for the 5-HT4 receptor, vectors, host cells, probes, antisense nucleic acids, and a method for detecting expression of a mammalian 5-HT4 recept r, classified in at least, for example, Class 536, subclass 23.5.

II.Claims 38-39, drawn to a method of isolating a gene, classified in at least, for example, Class 435, subclass 172.3.

III.Claims 43-44, drawn to a 5-HT4 receptor protein, classified in at least, for example, Class 530, subclass 350.

IV.Claims 45-48, drawn to a method of production of a mammalian 5-HT4 receptor, classified in at least, for example, Class 435, subclass 69.1.

V.Claims 49-54 and 61, drawn to an antibody to a 5-HT4 receptor protein, classified in at least, for example, Class 530, subclass 387.1.

VI.Claim 55, drawn to a pharmaceutical composition to alleviate abnormalities resulting from overexpression of a 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.

VII.Claim 56, drawn to a pharmaceutical composition to alleviate abnormalities resulting from underexpression of a 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.

VIII.Claims 57-60, drawn to pharmaceutical compositions containing oligonucleotides, classified in at least, for example, Class 514, subclass 44.

IX.Claims 62-66, drawn to transgenic animals, classified in at least, for example, Class 800, subclass 2.

X.Claims 67-68, drawn to a method for determining physiological effects of varying levels of 5-HT4 receptors using transgenic animals, classified in at least, for example, Class 514, subclass 44.

XI.Claims 69-70, drawn to a method for determining specific binding using whole cells, classified in at least, for example, Class 435, subclass 7.2.

XII.Claims 71-72, drawn to a method of screening compounds to identify drugs using whole cells, classified in at least, for example, Class 435, subclass 7.2.

XIII. Claims 73-74, drawn to a method for determining specific binding using cell extracts, classified in at least, forexample, Class 435, subclass 7.1.

XIV.Claims 75-76, drawn to a method of screening compounds to identify drugs using membrane fractions, classified in at least, for example, Class 435, subclass 7.1.

XV.Claims 77-82, drawn to a method for identifying a compound using whole cells and activation blockade, classified in at least, for example, Class 435, subclass 7.2.

XVI.Claims 83-84, drawn to compounds and pharmaceutical compositions, classified in at least, for example, Class 514, subclass 2.

XVII.Claim 85, drawn to a method for detecting 5-HT4 receptor on the cell surface using an antibody, classified in at least, for example, Class 435, subclass 7.2.

XVIII.Claim 86, drawn to a method for treating an abnormal condition related to excess activity of 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.

XIX.Claim 87, drawn to a method for treating an abnormal condition alleviated by increasing the activity of 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.

XX.Claims 88-89, drawn to a method for diagnosing a predisposition to a disorder associated with expression of 5-HT4 recept r alleles, classified in at least, for example, Class 435, subclass 6.

XXI. Claim 90, drawn to a method of identifying a substance that alleviates abnormalities from overexpression of 5-HT4 receptors using transgenic animals, classified in at least, for example, Class 424, subclass 9.

XXII. Claim 91, drawn to a method of identifying a substance that alleviates abnormalities from underexpression of 5-HT4 receptors using transgenic animals, classified in at least, for example, Class 424, subclass 9.

XXIII. Claim 92, drawn to a method of treating abnormalities alleviated by reduced expression of 5-HT4, classified in least, for example, Class 514, subclass 44.

XXIV. Claim 93, drawn to a method of treating abnormalities resulting from underexpression of 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.